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Modelling the transmission of gastrointestinal nematodes in saiga antelope under changing demography and climate.

Modelling the transmission of gastrointestinal nematodes in saiga antelope under changing demography and climate.

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Abstract

The saiga antelope, *Saiga tatarica*, is a critically endangered migratory antelope that exists in the steppe and semi-deserts of Central Asia. Saigas host several gastrointestinal nematode (GIN) species, which can potentially affect their fecundity and fitness. The environment that this host-parasite system inhabits is highly seasonal, with extreme peaks of temperature and low and stochastic precipitation. With a large portion of their life cycle spent outside of the host, different GIN genera have contrasting adaptations to facilitate transmission in these highly variable conditions. To persist, GIN must overcome long periods of migratory host absence and large fluctuations in host demographics.

In this study, a mathematical model was constructed and calibrated to estimate the basic reproductive ratio (R_0) of selected GIN species and examine how climatic factors affect their ability to propagate in these challenging conditions. The model was tested using climate and saiga demographic data from 1979 to 2017, and historical and new data on parasite prevalence and diversity in saigas in Kazakhstan, including those collected in the field in 2017 as part of the study.

Results suggest that transmission of *Nematodirus* and *Marshallagia* spp. benefit from seasonal aggregation of saigas, which maintains host density despite wide fluctuations in numbers and seasonal distribution, enabling parasite persistence. In contrast, R_0 of trichostrongylids (represented by *Haemonchus contortus*) was frequently below the threshold for persistence due to periods of low host density and unfavourable climatic conditions. These genera are likely to rely on livestock presence within the saiga range to support persistence through spill-over and spill-back events. Host demography was more important in driving parasite persistence than changes in climate over the studied period. Predictions were supported by available data on parasite presence in saigas and fieldwork carried out as part of this study. Implications for conservation of threatened saiga populations are discussed.

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Dan Comber – Whose advanced knowledge of Microsoft Excel helped greatly in growing my familiarity with this software.

Becky Randle – Whose emotional and advisory support helped me through the most challenging periods of this project.

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ..... DATE:.....

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1.0 Introduction

This study's aim was to build and modify a mathematical model in Microsoft excel based upon a model structure previously devised to study parasite transmission in Europe.

From simulated results, inferences are made regarding the success or failure of a select range of parasite genera, within the complex ecosystem described in this study. Parasite transmission is affected by climatic variables and host density. Consequently, the parasite genera have undergone adaptations to facilitate survival and reproduction based on these variables. Using theoretical scenarios based on reality, this study attempts to understand why certain life history strategies may have been selected for under the discussed variables. I conclude this thesis with predictions of what might happen to this ecosystem under potential future climatic conditions and further disruption to the potentially already unstable host-parasite dynamics of this system.

1.1 Background

Parasitic gastrointestinal nematodes (GIN) have the potential to significantly increase mortality rates and reduce reproductive success in both livestock and wildlife populations worldwide (Knox *et al.* 2006, van Houtert and Sykes 1996).

GIN spend a significant portion of their life cycle in the external environment outside their host, therefore the success of GIN species is subject to the variables of this environment. Each change in an environmental factor, be it due to gradual climate change or anthropogenic modification of an ecosystem, shifts the context within which parasites, their hosts and their vectors breed (Patz *et al.* 2000).

Climate change is a continuous driver of changes in parasite dynamics, generating functional and microevolutionary responses in response such as shifting patterns of geographic range, changes in gene frequencies and local extinction, which affect the structure of entire host-parasite communities (Brooks & Hoberg 2007). Changes in climate within and between years may lead to unexpected high intensity outbreaks at otherwise "safe" times of year, which has large implications for host condition (Fox *et*

al. 2014). Equally, should climate change reduce the frequency of ideal conditions for parasite survival in the external environment, global change carries an extinction risk for parasite species. In multi-parasite systems, the risk for each species will vary with life history strategy and traits (Cizauskas *et al.* 2017).

Many common ruminant hosts of GIN incorporate migration as part of their life history strategy. While migration may be primarily for reasons such as food/water availability or predator avoidance (Dingle *et al.* 2011), it is hypothesised that parasite avoidance may be a migration driver, where hosts vacate habitat to avoid parasite ingestion *(*Hall *et al.* 2016). Migration causes variation in host availability, a factor to which adaptations have developed in parasite species (Gibbs 1986a, Beaumont *et al.* 2009). There are multiple systems around the world involving GIN and a migratory host: such as reindeer/caribou in Canada and Svalbard (Irvine *et al.* 2011, Dobson *et al.* 2015), and wildebeest on the African plains (Mijele *et al*. 2016).

Understanding how parasite success changes over time and the factors that cause this change can provide information critical to the conservation of wildlife populations and maintenance of sustainable livestock agriculture. Examining individual host-parasite systems help to grow this understanding. This is the main aim of this thesis.

1.2 The Study

This study focusses on the host/parasite system of the Saiga antelope (*Saiga tatarica*) and an assortment of parasitic gastrointestinal nematode genera, within the steppe and desert environments of Kazakhstan.

1.2.1 The Saiga Antelope

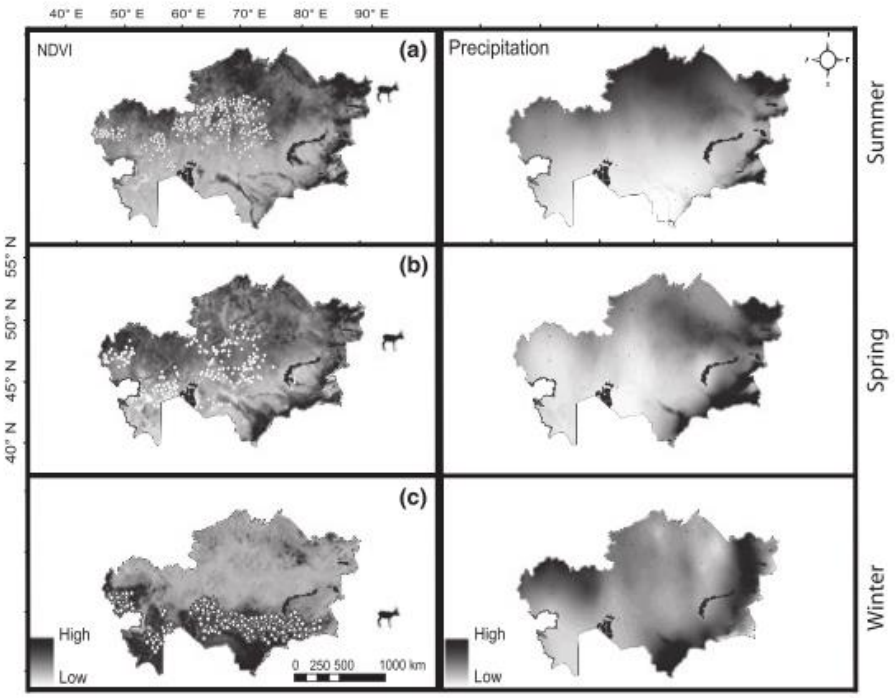
The Saiga antelope (*Saiga tatarica*) is an ungulate and ruminant of the family Bovidae. 4 populations of the subspecies *Saiga tatarica tatarica* and 1 population of the subspecies *Saiga tatarica mongolica* are extant. 3 populations of *S.t.tatarica* exist

within Kazakhstan; the “Ustiert”, “Ural” and “Betpak-dala”. The 4th population is located within the Russian province of Kalmykia, split from the Kazakhstan group by irrigation canals (Milner-Gulland 1994). *S.t.mongolica* is morphologically distinct and confined to a small population in Western Mongolia (Lushchekina & Dulamtseren 1997). This thesis will focus primarily on the Kazakhstan populations.

The saiga antelope is a seasonally migratory species. The migration route takes place latitudinally, following intra-annually and inter-annually varying gradients in vegetation productivity and precipitation (Singh *et al.* 2010a). The Saiga range is divided into 3 periods of Summer, Spring and Winter ranges. Overwintering takes place in the latitudinally southern winter range, in harsh temperature conditions albeit less harsh than at northern latitudes.

Saiga are herbivorous grazing animals; a large proportion – 22%-88% - of their plant intake comes from seasonally available flowering plant species from the genera *Kochia, Galatella, Tulipa and Artemisia* (Abaturov & Subbotin 2011). As temperatures and precipitation climb across all 3 ranges, vegetation production also increases. The latitudinally southern ranges see an increase in productivity first, although peak productivity within years is much larger in the Spring and Summer ranges, thus Saiga move latitudinally north from February – June to maximise food intake (Figure 1, Figure 2). The benefit of maximising nutritional intake and avoiding harsh climatic conditions offsets the high energy expense of the long-distance migration.

The growth of human settlements, agriculture and landmarks such as roads, railways and border fences have the potential to change or limit saiga migration paths (Bull *et al.* 2015), particularly in regions where the migration route consistently involves a bottleneck or small region as in the Mongolia population (Berger *et al.* 2008).



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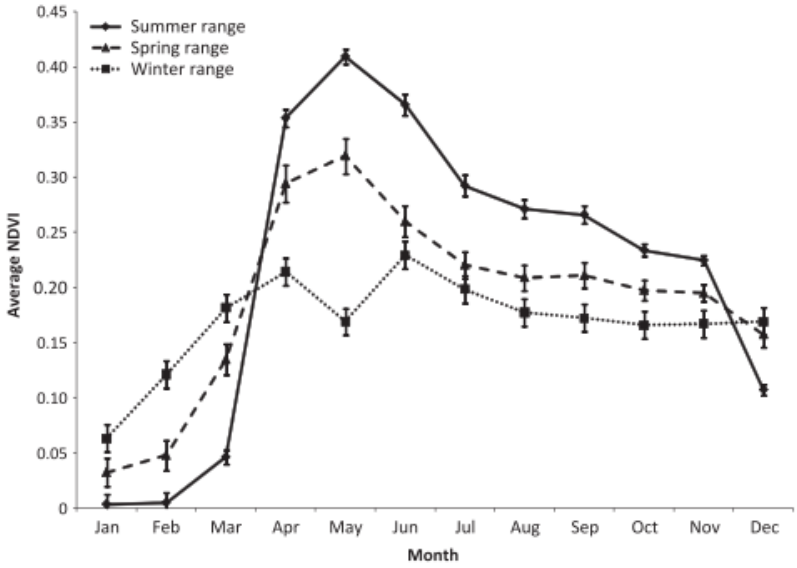


Figure 2) Singh et al. (2010). Annual trends of average normalized difference vegetation index (NDVI) representing vegetation productivity in the seasonal ranges of Kazakhstan saiga populations.

A key aspect of Saiga ecology is reproductive strategy. During the spring period of April – early June, saiga females aggregate in large numbers to undergo a mass calving taking place of a short period of 3-8 days (Fadeev and Sludskii 1982). The location of these aggregations varies annually due to several influences and factors (Singh *et al.* 2010b). Analysis revealed that like the overall migration route, calving site selection varies with vegetation productivity to maximise food intake, although consistent inter-annual productivity was preferred over potentially richer but more stochastically productive regions. Calving sites occur at a distance from a water source in a trade-off between closeness to the water source and increased risk of predation and disease due to the closeness to said source. Additionally, there is a human influence, with the distance between calving sites and settlements having doubled in recent decades.

Mapping saiga calving locations by decade reveals a northwards trend in location choice (Figure 3), potentially indicative of a changing climate resulting in milder winters and an earlier boom in summer vegetation productivity (Singh *et al.* 2010b).

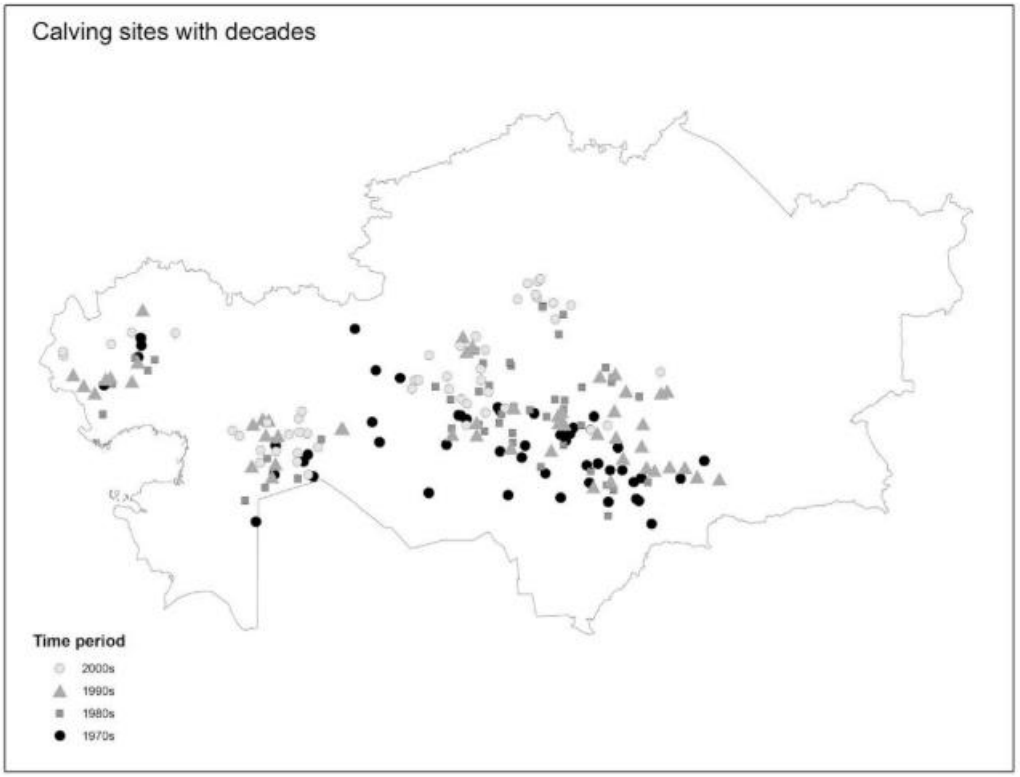


Figure 3) Singh et al. (2010) Saiga antelope Spring aggregation calving sites in Kazakhstan by decade from 1970's to 2000's. 2000's = Light circle, 1990's = Triangle, 1980's = Square, 1970's = Black circle.

The future of these aggregations is not guaranteed. Estimates from aerial surveys have suggested the reduction in calving aggregation frequency, particularly in the Ustiert and Kalmykia populations (Fry 2004). Institute of Zoology survey reports note reduced aggregation frequency in all 3 Kazakhstan populations due to the population declines and increased human disturbance (Kühl *et al.* 2009, Singh *et al.* 2010b).

1-2 days following birthing, females collect their calves and continue the migration. For this to occur, neonates are born large and highly developed; unusually so compared to the size of the adult female. This is made possible by massive reproductive investment by female saiga during gestation. The investment is not inconsequential, with resources diverted towards foetuses, immunological functions are weakened; an example of a seasonal trade-off between reproductive success and individual survival (Nelson 2004). The weakening of saiga females leading up to calving has been proven to be relevant to disease transmission (Kock *et al.* 2018) and is likely to be relevant to GIN success.

Additional aggregations of Saiga occur in the same geographical Spring range as calving, during the Autumn period. During this time males enter the rut, competing for harems of females (Milner-Gulland *et al.* 2003).

Once ubiquitous across Eurasia, hunting and severe “dzhuts” – extremely harsh winters with extensive, long-lasting snow cover – reduced the Saiga population to an estimated few hundred individuals by 1930. Hunting for meat, horns and hide had been a large factor in decline leading up to the 20th century (Lushchekina & Struchkov 2001). The horns historically, and at present, carry great value in east-asian markets, particularly China. This drives demand for poaching in the Saiga regions (Chan *et al.* 1995). A Soviet ban on hunting of the animal and a series of mild winters saw a recovery to 1 million individuals by 1954 and 2 million by 1973 (Stoddart 1974).

A series of mass mortality events due to dzhuts and pasteurellosis caused a drop from 480000 to 250000 individuals in the 1980’s (Bekenov *et al.* 1998). Following the collapse of the Soviet Union, desperation driven overhunting caused a 90% decline in population size (Milner-Gulland *et al.* 2003). Protection efforts generated substantial

population recovery during the early 2010's. However, in the duration of 3 weeks of May 2015, a mass mortality event resulted in a 62% loss of the Betpak-dala Saiga population. An investigation concluded that the bacterium *Pasteurella multocida* had caused the mortality, but as the normally commensal organism only becomes lethal opportunistically with weakening of the host immune system, other factors must have been involved (Milner-Gulland 2015).

Multivariate analyses linked climatic conditions to mass-mortality events, showing strong but incomplete differentiation in temperature and humidity between sites at which mass-mortality events took place and sites where Saiga remained unaffected. The scale of these mortality events may be a result of the previously mentioned high investment in reproduction leading to weakened immune systems in the female population during the calving accumulations (Kock *et al.* 2018).

1.2.2 Kazakh Region

The Central Asian Steppe ecosystem is defined as a highly stressed environment with polarised extreme temperatures in winter and summer months, and stochastic rainfall (Sapanov 2018).

The region of Central Asia can be referred to as a climate hotspot (Turco *et al.* 2015). In comparing climatic records of consecutive periods, modern (1981-2010) and past (1951-1980), the criteria of 7 climate change indicators are considered when applying this term:

1) Absolute change in mean temperature.
2) Percentage change in mean precipitation with respect to the mean over 1951-1980.
3) Percentage change in interannual standard deviation of the detrended temperature.
4) Percentage change in the interannual coefficient of variation of the detrended precipitation.
5) Frequency of seasons with temperatures exceeding the temperature maximum in 1951-1980.

6) Frequency of seasons with precipitation exceeding the precipitation maximum in 1951-1980 (f_{wet}).
7) Frequency of seasons with precipitation below the minimum seasonal precipitation in 1951-1980 (f_{dry}).

The modelling efforts based on these criteria, by Turco *et al.* (2015) identified central Asia as a hotspot of climate change, along with large areas of Africa, Indonesia and the Amazon; in particular when only accounting for criteria 1-4. Temp changes are strongly significant and criterion 2 is significant but only for winter months.

In respect to precipitation, the key factor is not the length of droughts but their frequency. While Kazakhstan suffers droughts lower in intensity and length than the neighbouring nations of Tajikistan, Kyrgyzstan and Uzbekistan, the frequency of these droughts is high (Figure 4).

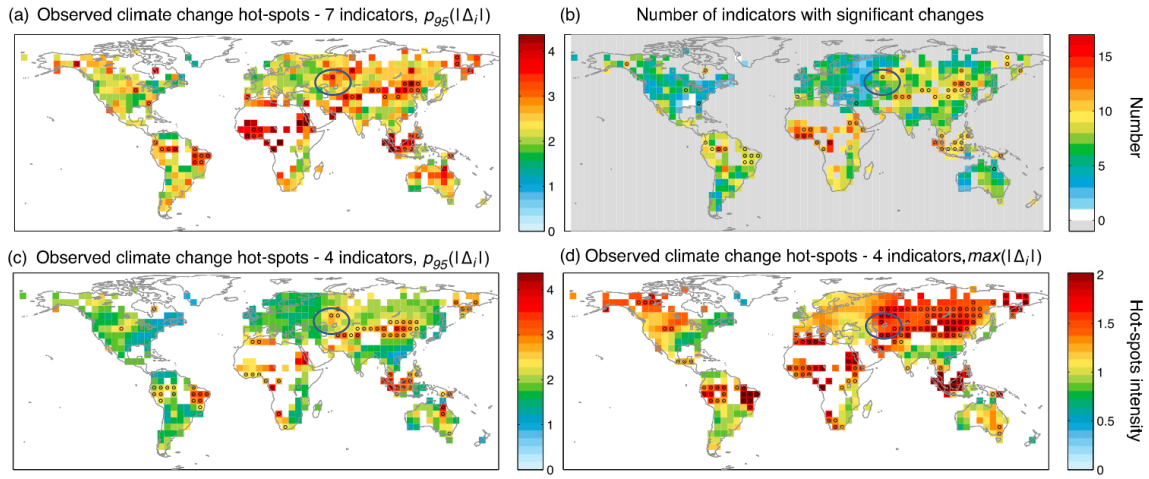


Figure 4) Turco et al. (2015) a) Observed climate change hotspots using all 7 indicators and normalisation factors p_{95}; (b) number of climate indicators that show statistically significant change; (c) hotspots considering only change in temperature, change in temperature variance, frequency of seasons with temperatures above the past maximum seasonal temperature and change in precipitation; (d) as (c) but with global maximum of field as normalisation factor. Black circules indicate significance (95%). The central Asia region is outlined by a blue oval.

The human effects on the Kazakhstan region differ with land usage and human density. As the country is sparsely populated, the most notable effects are from agricultural land use. Agriculture has been present in Central Asia (particularly Kazakhstan) since 3000BC (Spengler *et al.* 2016).

The conversion of large areas of land from steppe to cropland has directly affected some regions. Most significantly due to the diversion of water body tributaries for cropland irrigation. The most notable example is the near total desiccation of the Aral Sea towards the end of the 20th century; a reduction of the lake's surface area by 74% had great ecological consequences – including rapid climate change in the vast areas of land previously bordering the lake or submerged by water (Micklin 2007).

The keeping of animal livestock is extremely common. While cattle are present, sheep/goats are by far the most numerous animals bred for slaughter in the region. In the pre-soviet era livestock movement was unrestricted and covered a larger area. This area became more limited as Soviet policy supported domestic livestock with feed during the winter and thus removing the need for migration to productive pastures. The dissolution of the Soviet Union saw a crash of livestock numbers and famine that drove up saiga hunting during the 90's, as well as reduced veterinary care for the remaining livestock population (Robinson & Milner Gulland 2003, Lundervold *et al.* 2004).

Within the saiga ranges today livestock populations typically are kept near the villages and farms to which they belong. Regardless, the sharing of pasture between saiga and livestock is possible. This sharing of pasture has consequences for parasite transmission in this scenario, with multiple parasite genera shared between livestock species and saiga. Models have demonstrated the potential for parasite transmission between these populations, especially within the Winter range where both saiga and livestock share limited pasture space (Morgan *et al.* 2006). The implications of this transmission potential are discussed in the further sections of this thesis.

1.2.3 Parasite Species

Thirty-eight helminth species are known to infect saiga or ruminant livestock (sheep and goats) in Kazakhstan (Morgan et al. 2005). Previous research on this system (Morgan *et al.* 2005, 2007) has identified 3 key genera as the most suitable for study. *Haemonchus, Marshallagia* and *Nematodirus* are selected as the focus of this study because of their ubiquity both in this ecosystem and others worldwide, the relatively high damage they cause to their hosts, and distinct, well understood life history strategies. Similarities and differences between these species are shown in (Figure *5*) and are discussed in the following section.

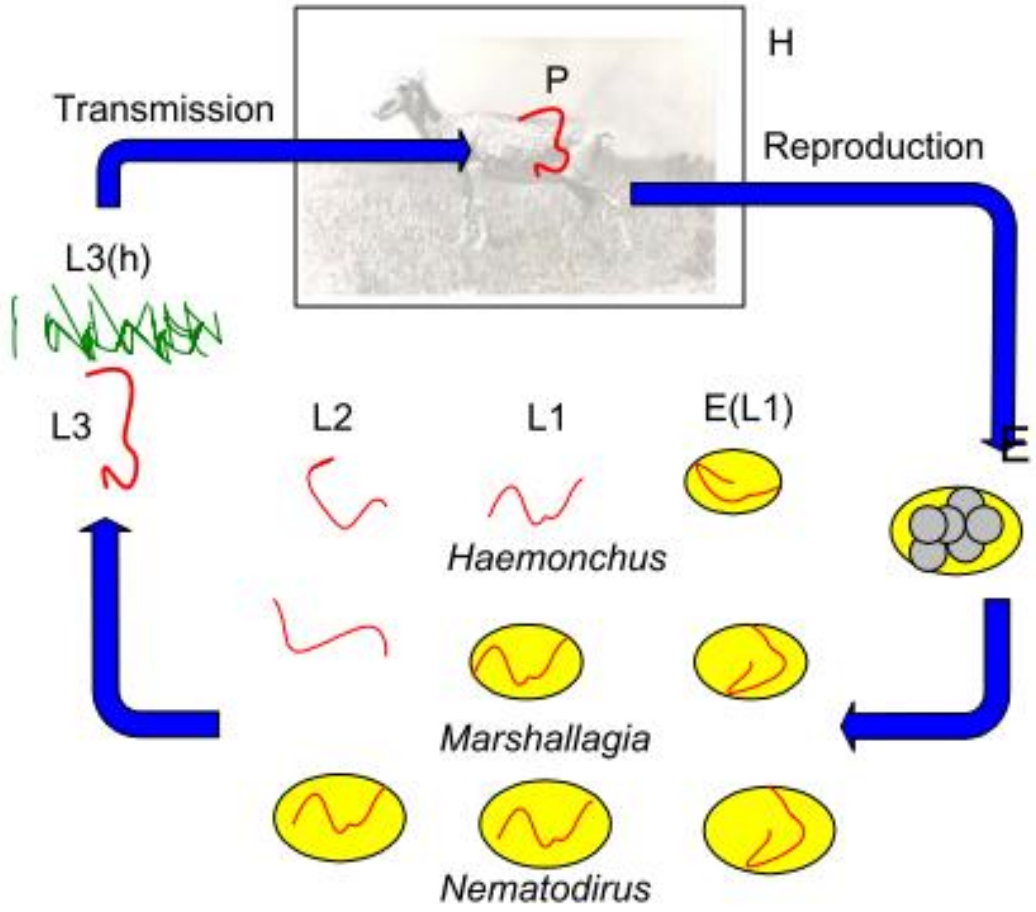


Figure 5) Morgan et al, (2005) GIN life cycle, showing difference between genera. E(L1) E=eggs, E(L1)=first stage larva in the egg and ready to hatch, L1-3=first, second and third stage larvae, L3(h)=infective, third stage larvae on the herbage, P=adult worms, H=host.

Genus Haemonchus

The genus *Haemonchus* is globally widespread; present on all continents with exception of Antarctica. *Haemonchus* species infect a diversity of hosts, but most critically, domestic sheep and goats that make up one of the worlds most common food sources. Haemonchosis disease in livestock can be highly damaging if left untreated and represents a threat to agriculture and livelihood. Likely due to its effect on humanity, the life history and ecology of *Haemonchus* species is well understood, with comprehensive studies carried out in the United States, South Africa, Australia, Europe and across Asia (Silverman & Campbell 1959).

Haemonchus contortus is by far the species least robust to external environmental factors out of those discussed in this thesis. Particularly, the pre-infective stage is highly susceptible to extremes of both hot and cold temperature. *Haemonchus contortus* is completely reliant on the presence of moisture for free-living development, remaining extremely vulnerable to desiccation until the infective stage is reached; at which point individual survival under dry conditions is slightly increased (O'Connor *et al.* 2007).

The comparatively low investment and low survivability of eggs and early larval stages is offset by high fecundities of thousands of eggs per day produced by each *Haemonchus contortus* adult female (Coyne *et al.* 1991); in comparison to a fecundity of <100 eggs dy^{-1} in *Nematodirus* and *Marshallagia. Haemonchus* is classic example of an r-selected species that favours high fecundity and rapid growth at the cost of extended individual survival rates.

The lack of survivability due to extreme environmental conditions should limit *Haemonchus* to the temperate climates that it is known to succeed in. However, the spreading of its distribution into colder regions (Waller *et al.* 2004) and regions of frequent aridity (El-Azazy 1995) suggests adaptation.

The limited ability of *Haemonchus* eggs to survive for long periods in an external environment is offset by a strategy to avoid challenging environmental conditions. *Haemonchus* species are known to employ hypobiosis (Blitz & Gibbs 1972). "Hypobiosis" refers to the arrest of a final larval stage upon ingestion by the host, for a short or prolonged period of time. After the elapse of this period or when an

alternative trigger is present, the individual ceases arrest and continues development to the sexually mature stage (Gibbs 1986a). An individual ingested in Autumn may arrest development over the unfavourable winter months, beginning egg production in the Spring.

In the case of *Haemonchus* whose primary hosts are ruminant livestock and wild animals that birth in the Spring, an increase in egg production during this period and the termination of hypobiosis is known as the periparturient rise (Gibbs 1986b). The drop in host immunity due to hormone changes within lactating females diminishes the factors limiting GIN egg production. Not only does this strategy allow avoidance of winter conditions but also induces maximum egg output simultaneous with maximum naïve host availability (Courtney *et al.* 1984).

Despite in theory being ill suited to a dry climate or regions where precipitation is highly variable, *Haemonchus sp.* are known to have been present in Kazakhstan livestock populations (Morgan *et al.* 2005).

Genus Nematodirus

As with the other 2 genera focussed on in this study, *Nematodirus* has a widespread geographical distribution, affecting well studied wildlife and livestock populations across the globe. Like *Marshallagia* and unlike *Haemonchus,* members of the genus *Nematodirus* are incredibly resilient to damage done by environmental conditions and have a high survival rate throughout their external life cycle. An adaptation of this genus to allow this is that the entirety of larval development from egg to infective L3 takes place within the protection of the egg (Thomas 1959), whereas *Haemonchus* and *Marshallagia* hatch at L1 and L2 respectively.

Another key adaptation employed by some, but not all members of the *Nematodirus* genus is called “bet-hedging”. Bet-hedging is “the stochastic switching between phenotypic states, an evolutionary adaptation that facilitates persistence in the face of fluctuating environmental conditions” (Beaumont *et al.* 2009). It is evolutionarily beneficial under the presence of unpredictable conditions such as variable climatic conditions or variable timing of host availability. Spreading the availability of offspring

over an extended time period increases the probability that at least a proportion greater than 0% of offspring will be transmitted, at a trade-off of the loss of opportunity for maximum transmission of offspring (Cable *et al.* 2017). In the case of *Nematodirus* bet-hedging involves the delaying of hatching following the completion of development to infective L3 within the egg (Thomas & Stevens 1960).

Some species in this system are known to have bet-hedging, while some do not. *N.battus* shows varying extents of bet-hedging within species. A study obtaining isolates of *N.battus* from different regions across the United Kingdom showed that in the cooler climate of Scotland where climatic conditions in Spring are consistently good for hatching, low proportions of eggs hatched without chilling, whereas in Bristol where the window of opportunity for Spring transmission is much smaller, delaying hatching overwinter could result in missing the sharp increase in Spring temperatures through this window. The Bristol isolate therefore showed a lower proportion of bet-hedging (van Dijk & Morgan 2010).

A New Zealand study on *N.filicollis* and *N.spathiger* reported that the former species shows hatching at 72% after 33 days of chilling at 4^{o}c (requiring this chilling for development), and the latter required no chilling whatsoever (Oliver *et al.* 2016). The latter species could develop to infective L3 in ideal conditions immediately following introduction to soil. *N.filicollis* benefits by timing egg development to ideal environmental and host presence conditions in the Spring, whereas *N.spathiger* can produce multiple generations of infections within a single year. The differences in strategies between species within the same environment could be due to interspecific competition.

Additionally, anthropogenic effects may select against bet-hedging, for example in the fish louse *Argulus foliceus* the proportion of individuals exhibiting bet-hedging is lower in fish farms where host presence is guaranteed than in wild hosts (Pasternak *et al.* 2000). In the case of this system, *Nematodirus* species transmitting amongst a Kazakh livestock population might benefit from no bet-hedging.

Genus Marshallagia

Within the *Marshallagia* genus, the species most relevant to this study is *Marshallagia marshalli,* due to its abundance in the Kazakh region (see later). *Marshallagia marshalli* is a member of the family Ostertagii.

M.marshalli has a latitudinally widespread distribution stretching from the deserts of north Africa and the middle east, through the steppe of Eurasia and into the arctic (Hoberg *et al.* 2012), and a diverse assortment of host species – primarily ruminants – including numerous antelope species, camels *Camelus spp.*, sheep/goats *Ovis spp./Capra spp.*, cattle *Bos taurus*, red deer *Cervus elaphus*, reindeer *Rangifer tarandus* (Suarez & Cabaret 1991). Recent research however reveals a specialist life history strategy including significant resistance to cold and desiccation. Infective stage 3 larvae are protected by a sheath based on the second stage cuticle, which reduces the damage caused by adverse climatic conditions (Carlsson *et al.* 2012).

Unlike other Ostertagiines of both wild and domestic ruminants that are found mostly in temperate climates, *Marshallagia* is discriminated by climate towards steppe or temperately colder environments (Cabaret 1991).

Marshallagia marshalli is somewhat unusual in its usage of the sub-freezing temperature winter months to carry out transmission as first observed by the overwinter increase in worm infection abundance in Svalbard Reindeer (Halvorsen *et al.* 1999). Despite a peak worm burden in summer, high survival in cold conditions leads to eggs persisting on the ground, infecting hosts through the limited pasture available or by coprophagy. Later research demonstrated a significantly increased *Marshallagia* worm burden at the end of Winter, in hosts treated with anthelmintics in the prior Autumn (Carlsson *et al.* 2012).

Further analysis of worm burdens in Svalbard reindeer across years showed that the pattern of increase in *M.marshalli* did not vary between years (Irvine *et al.* 2000). This suggests that the population is not as strongly influenced by the external factors (such as climate and host density) as other members of the family such as *Ostertagia gruehneri.*

The environmental conditions of Svalbard show similarities to Kazakhstan, harsh environmental conditions are present for a large portion of the year, precipitation may become limited, host location changes throughout year with some extent of predictability. The difference lies between the summer conditions, the Svalbard summer is cool and temperate with adequate rainfall, in opposition to the extreme heat and frequent desiccation of the Kazakh steppe summer. The ability of *Marshallagia* to resist cold is well acknowledged, but slightly less so is the ability of eggs or post-hatching larval stages to resist extreme high temperatures and removal of water.

Studies on *M.marshalli* infecting livestock populations in Saudi Arabia suggest that like *Haemonchus,* arrested development is employed during the Spring so that ingested larvae are not sending eggs into a hot, dry environment (where their chances of survival and transmission are limited) (El-Azazy 1995). This contrasts with Svalbard, where authors specify the diminished involvement of arrest in the life cycle. It is likely that *Marshallagia* species develop adaptations specific to their local environment (Irvine *et al.* 2000).

The limitation of the low availability studies and uncertainty over the definition of *Marshallagia* species may mean that *Marshallagia* demonstrates a multiple variation of sets of adaptations across its large geographical range. This thesis can consider only the information presently available.

It is noted that a second species – *Marshallagia mongolica* – is present in the steppe ecosystem and is known to infect Saiga (Morgan *et al.* 2005) and the Mongolian gazelle *Procapra guttorosa* (Kuznetsov *et al.* 2014). However, this species remains even less quantitatively and qualitatively studied than *M.marshalli* and there is some debate about whether the 2 species are distinct. In this study only *M.marshalli* is considered.

1.2.4 Modelling Efforts

Mathematical models have a history of advancing understanding of host-parasite dynamics, in tandem with field and laboratory studies (Grenfell *et al.* 1995, Morgan *et al.* 2004).

Since the development of previous mathematical studies involving the study of this system (Morgan *et al.* 2005, 2007), information about parasite species has changed (Van Dijk & Morgan 2008, 2009, Rose *et al.* 2015, 2016, Carlsson *et al.* 2012) better models have been built to model parasite transmission (Bolajoko *et al.* 2015, Rose *et al.* 2015) but have only been applied to western temperate environments, saiga demography has shifted in the recent decade and their migration is more comprehensively understood (Singh *et al.* 2011).
We seek to gain further understanding of how parasites persist and why, as the climate and demography changes over time, finally making some predictions about the future.

Mathematical models are employed here in an attempt to disentangle the previously discussed factors affecting the transmission success of difference GIN genera and to make predictions about which life history strategies are likely to succeed or fail in this specific ecosystem.

Using these models, the aims are to make inferences about:

- Which parasite genera succeed or fail in this environment, under varying climatic and demographic scenarios?
- Which life-history strategies support parasite persistence and why?

1.2.5 Why is it important to study this system?

The conclusions of this study have the potential to contribute towards the conservation of the Saiga antelope, a critically endangered keystone species and an important part of the steppe ecosystem. Conclusions may also be used in the study of other host-parasite systems involving harsh climatic conditions or a migratory host.

This work will make inferences not only on parasite transmission in a steppe ecosystem but in similar ecosystems globally where GIN's exist, affecting wildlife and livestock populations.

2.0 Methodology

2.1 Modelling Background

Overall, the modelling efforts utilised in this study follow the structure of the basic reproduction quotient for macroparasites (Q_0). Q_0 was first devised as the basic reproduction quotient for macroparasites in Roberts & Heesterbeek (1995) as a way of providing an index of infection pressure of macroparasite populations. Q_0 estimates the average number of second-generation mature adult worms produced by a single adult worm during its lifetime in the absence of density dependent constraints – such as immunity and within-host competition (Bolajoko *et al.* 2015).

The Q_0 model can be defined as an equation and separated into 2 distinct components (Rose *et al.* 2016):

A) The number of L3 produced by an adult worm during its lifetime.

B) The number of adult parasites produced by each L3, as a function of L3 survival on pasture, the rate of intake of pasture by L3 of individuals hosts, and host density.

$$Q_0 = \underbrace{\frac{q\lambda}{\mu}}_{A}\underbrace{\frac{\beta p}{\rho + \beta H}}_{B}H$$

The symbols of this model equation are defined in Table 1, with exception to q (which refers to the probability of egg development to L3 under varying climatic factors). These climatic factors have an impact upon development (δ) and mortality (μ).

$$q = \frac{\delta m_1}{(\mu_e + \delta)(\mu_{l3} + m_1)}$$

Table 1 List of model parameters for Microsoft excel based model, based on model parameters in Rose et al. (2016) Q_0 model.

Symbol	Parameter	Unit
T	Daily Average Temperature	^{o}c
P	Total Daily Precipitation	mm dy^{-1}
TD	Difference between weekly max and minimum temperature	^{o}c
Ra	Extra-terrestrial Radiation	mm dy^{-1}
KT	Empirical Coefficient	-
ET0	Daily Potential Evapotranspiration	mm dy^{-1}
N	Saiga Population Size	-
λ	Fecundity	eggs dy^{-1}
δ	Daily development rate from egg to L3	-
δ_E	Daily development rate from egg to intermediate larval stage	-
δ_h	Daily egg hatch rate (if not incorporated into δ_E or δ_L)	-
δ_L	Daily development rate from intermediate larval stage to infective larval stage (L3)	-
μ_E	Daily mortality rate of eggs	-
μ_L	Daily mortality rate of intermediate larval stages	-
μ_{L3}	Daily mortality rate of infective stage larvae	-
μ_{L3p}	Daily mortality rate of infective stage larvae on pasture	-
m_1	Migration rate of infective larvae from faeces to pasture	-
c	Daily Herbage Dry Matter Intake per host	kg Dm ha^{-1}
B	Biomass	kg DM ha^{-1}
Area	Host Area Range (Variable by season)	ha
H	Host Density	N ha^{-1}
β	Rate of ingestion of L3 on pasture (c/Barea)	-
ρ	Probability of establishment of ingested L3	-

The original primary objective of modelling efforts was to apply Q_0 in statistics software R. However, the unsuitability of this model structure to study GIN species with life cycles involving an extended time period or periods of developmental arrest in the external environment, led to a change in the research direction of this study.

Regardless of this shift in model direction partway through this study, a series of maps showing basic average Q_0 ranges, produced in R, over a huge range of Kazakhstan gridpoints is included for the species *Haemonchus contortus.* These maps test purely the effect of climatic conditions on Q_0 with no regard to demographic variables, unlike the majority of modelling efforts in this study which include both.

To better meet the aims of this study, a model using the structure of the Q_0 model was produced in Microsoft Excel. It follows a framework based on the dismantled components of the Q_0 equation. This is similar to previous modelling efforts in that eggs inputted into the model at a constant rate advance through developmental stages and host ingestion at rates dependent on climatic and demographic factors (Table 2).

Table 2 Series of model equations regarding movement of individuals between stages of macroparasite life cycle, that form the structure of the excel model.

Life Cycle Stage	Equation referring to the number of individuals at any life cycle stage on a single day (d).
Eggs (E)	$E^d = E^{d-1} + \lambda - (E^{d-1} * \delta_E{}^{d-1}) - (E^{d-1} * \mu_E{}^{d-1})$
Intermediate Larval Stage (L)	$L^d = L^{d-1} + (E^{d-1} * \delta_E{}^{d-1}) - (L^{d-1} * \delta_L{}^{d-1}) - (L^{d-1} * \mu_L{}^{d-1})$
Infective Larval Stage (L3)	$L3^d = L3^{d-1} + (L^{d-1} * \delta_L{}^{d-1}) - (L3^{d-1} * m_1{}^{d-1}) - (L3^{d-1} * \mu_{L3}{}^{d-1})$
Infective Larval Stage on Pasture (L3p)	$L3p^d = L3p^{d-1} + (L3^{d-1} * m_1{}^{d-1}) - (L3^{d-1} * \beta^{d-1}) - (L3p^{d-1} * \mu_{L3p}{}^{d-1})$
Ingestion rate of L3p	$IL3p^d = (L3^{d-1} * \beta^{d-1}) * H$
Establishment of Ingested L3p	$IL3p^d * \rho$
R_0	Sum of all ingested L3 produced by a single female over the elapsed time period.

The columns of the model form “chambers” containing the total number of individuals at each life history stage during any single time. The rows of the model represent time, with each consecutive cell forming a single day, from 01/01/1979 to 31/12/2017.

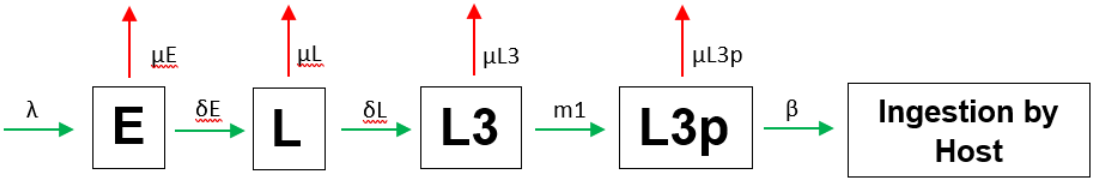


Figure 6 Structure of Microsoft Excel based R0 model. E = eggs, L = Intermediate stage larvae, L3 = infective stage larvae, L3p = infective stage L3 larvae on pasture, λ = Fecundity, δ = development between respective life history stages, m1 = migration rate to pasture, μ = mortality rate of respective life history stages, β = rate of ingestion of L3 on pasture.

This new model does not calculate Q_0, as the model does not consider further development to the adult stage following ingestion as in part B of the Q_0 equation. Rather, the output of this model estimates the number of eggs produced by a single female worm over the course of its lifespan that develop successfully to the infective L3 stage and infect a host. This is a variation upon R_0, the basic reproduction rate. R_0 calculates the expected number of secondary cases produced in a completely susceptible population by a typical infected individual during its entire period of infectiousness (Diekmann *et al.* 1990). While not identical to Q_0, this model structure is still capable of providing a threshold quantity to predict the ability of GIN populations to increase (R_0>1) or decrease (R_0<1) (Cross *et al.* 2007).

The advantage of the excel model is that the separation of the life history stages into different compartments allows clear examination of a numerical breakdown of each life history step over time. It is possible to view which life-history stages are graduated through quickly or gradually by the available individuals; and furthermore, when R_0<1, it is possible to identify the stage(s) of the life history strategy that individuals are unable to pass and therefore the conditions causing transmission failure.

In this study, the importance of broad observations is noted. A common theme throughout the parameterisation of this model is the use of quantitative data from a majority of highly controlled laboratory-based studies that themselves may not be representative of how development and mortality occur in a more stochastic natural environment. Due to the amount of estimation involved in modelling efforts such as this one, focussing on exact numerical outputs is likely to lead to inaccurate conclusions. Rather, the conclusions we attempt to draw are at a much broader scale that is appropriate to the level of accuracy provided by such mathematical models.

2.2 Model Parameterisation

2.2.1 Obtaining and usage of climate data

All climatic variables used in this model (Table 4) were obtained when possible or calculated in the absence of available data.

To describe the climate-driven temporal variation in transmission success over the Kazakhstan region, a gridded dataset of temperature and precipitation data, 2m above ground level, at a temporal resolution of 1 day was obtained from KNMI Climate explorer (climexp.knmi.nl). The dataset is formed from ERA-interim reanalysis data between the periods of 01/01/1979 and 30/06/2017. In the original Q_0 modelling efforts, the full range of the gridded dataset is used in mapping efforts. In the latter R_0 excel model that uses fixed saiga range sizes, specific individual grid points were chosen to represent the climatic conditions over the entire range size.

Ideally, it would be most beneficial to average all grid points over the approximate saiga seasonal ranges. While this averaging would be appropriate for temperature, the high variability and low levels of precipitation within years leads to a continuous extremely low average precipitation over the course of the year. This is not representative over the brief periods of high rainfall in an otherwise arid environment, observed in this system.

The climate data gridpoints chosen to represent each of the saiga ranges, in latitude x longitude, are:

Summer: 49.474x66.797

Spring: 47.368x67.500

Winter: 44.561x67.500

The unavailability of weather station climate data over the required time period at the time of this study necessitates the usage of reanalysis data. The full methodology behind the calculation of the climatic data used here can be found in Berrisford *et al.* (2011). There has been some debate over the creation of ERA reanalysis datasets (regarding the accuracy of the dataset, particularly regarding precipitation) where in previous ERA versions there have been problems with over or underestimation of precipitation over oceans or arid areas. The latter of which is relevant to this study. However recent strides in development and validation of ERA-reanalysis models using real weather data has improved accuracy and reliability (Dee *et al.* 2011). The climate data was found to be satisfactory with the suitability of this climate data for usage in this model.

This study also recognises that in practice the air temperature at 2m above ground level might not correspond exactly with the temperature experienced by eggs and early larval stages in soil and faecal matter, which itself will be variable with multiple factors. These factors include soil moisture content and the incidence of solar radiation on the soil. However, in the absence of data for daily soil temperatures over the region of Kazakhstan, the best alternative has been employed.

2.2.2 Calculating evapotranspiration

The R based Q_0 model includes a function to insert a latitudinally variable value of solar irradiance to calculate daily rate of evapotranspiration, a function relevant for parasite transmission. In the Microsoft excel based model, a novel method was required to replicate this function. For each latitude, a monthly value of solar radiance was obtained for each month of the year. Each set of monthly averages were averaged

over the series of latitudes dependent on saiga range: North = Lat 48-52, Centre = Lat 46-50, South = Lat 43-47 (Table 3).

Table 3 Average values of solar irradiance (Ra) calculated by latitude across the seasonal saiga ranges.

	North	Centre	South
Month	Solar irradiance Ra (mm dy^{-1})		
Jan	3.598	3.968333	4.84
Feb	5.828	6.2	7.058
Mar	8.99	9.318333	10.064
Apr	12.802	13.02167	13.506
May	15.698	15.795	15.998
Jun	17.076	17.09833	17.134
Jul	16.516	16.56667	16.666
Aug	14.156	14.315	14.662
Sep	10.598	10.88	11.514
Oct	6.99	7.346667	8.166
Nov	4.222	4.593333	5.462
Dec	3.08	3.445	4.31

To calculate evapotranspiration from solar irradiance, the Hargreaves-Samani equation for reference crop evaporation (ET_0) was utilised:

$$\mathbf{ET_0 = 0.0135(KT)(Ra)(TD)\ ½\ (TC+17.8)}$$

Where $TD = T_{max} - T_{min}$ (^{o}c) over a monthly period, Ra = solar irradiation, TC = average daily temperature over the same monthly period (^{o}c), and KT = empirical coefficient (Hargeaves and Samani 1985, Samani 2000).

KT is an adjustment factor for interior or coastal regions to account for proximity to a large body of water and elevation effects on atmospheric volumetric capacity (Samani 2000).

As Kazakhstan has a continental climate, the interior function used was based on that calculated for the continental United States (Samani 2000):

KT = 0.00185(TD) 2 - 0.0433 TD + 0.4023

Table 4 Parameterisation – List of climatic variables relevant to the development and mortality of GIN used in the model.

Symbol	Parameter	Value	Unit	Source
T	Daily Average Temperature	Daily Variable	^{o}c	KNMI ERA data
P	Total Daily Precipitation	Daily Variable	mm dy^{-1}	KNMI ERA data
TD	Difference between weekly max and minimum temperature	t_{max}-t_{min}	^{o}c	KNMI ERA data
Ra	Extra-terrestrial Radiation	Variable with latitude.	equivalent evaporated water depth (mm dy^{-1})	Santa Clara University calculation tool
KT	Empirical Coefficient	$KT=0.00185(TD)^2$ - 0.0433(TD) + 0.4023		Samani (2000)
ET0	Daily Potential Evapotranspiration	$ET_0=0.0135(KT)(Ra)(TD)^{1/2}(T+17.8)$	mm dy^{-1}	Samani (2000)

2.2.3 GIN Life History

As outlined in the model structure, individuals pass through the stages of the model at varying daily rates. These rates are dependent on the environmental and demographical variables that change over time. For each genus, rates were taken or calculated depending on available literature.

Rates regarding development and mortality throughout the life cycle of *Haemonchus contortus* were comprehensively reviewed and calculated in Rose *et al.* (2015, 2016). These studies reviewed the extensive collection of available experimental data across the 20th and 21st centuries. No further data is available regarding *Haemonchus contortus* transmission since these reviews, and therefore rate equations from these papers have been used directly in this model, with any exceptions outlined.

Marshallagia marshalli is the least understood of the 3 genera studied. Modelling attempts in the mid 2000's (Morgan *et al.* 2005, 2007) estimated approximate development and mortality rates across the life cycle. The sparse literature regarding this species since then has focussed primarily on distribution and life history traits, including no useful qualitative data for analysis. Therefore, parameters for development and mortality for this species have been taken from the above-mentioned papers, with modification when required.

Within the genus *Nematodirus, Nematodirus gazellae* is perhaps the most relevant species in terms of the saiga antelope as a host. However the relative isolation and insignificance to humans of this species mean quantitative data is totally lacking. By far the most understood species is *Nematodirus battus,* whose impact on livestock in the western world has led to extensive quanitative studies. However, the lack of *N.battus* in the Kazakhstan ecosystem, and its favourability toward temperate or tropical climates make pure *N.battus* rate equations unsuitable. *Nematodirus filicollis* is present in Kazkhstan saiga and livestock populations, and its life history was quantitatively investigated in Van Dijk & Morgan (2009), Oliver *et al* (2016). Quantitative information extracted from *N.battus* and *N.filicollis* papers were combined and averaged to form a series of rate equations representative of the genus *Nematodirus.*

Whether these rate functions were taken from other studies or calculated in this study, extensive time was taken to fully understand the reasoning for each of these rates in terms of GIN ecology and the experimental data used. These are discussed in detail in the following breakdown.

2.2.4 Fecundity

Previously discussed is the tendency of *H.contortus* towards a r-selected life history strategy and *M.marshalli* and *N.sp* towards a K-selected life history strategy. *H.contortus* investment in individual eggs is low, but is offset by the mass production of 5500 eggs dy^{-1} by an individual female (Coyne & Smith 1992).

Estimates of fecundity of *M.marshalli* and *N.sp.* in saiga and livestock were produced in (Morgan 2003) by comparing data of faecal egg output in experimental animals to the number of worms recovered from these animals after their slaughter. There is a linear relationship between faecal egg counts and adult worm burden. Fecundity of *M.marshalli* was estimated to be 98 eggs dy^{-1}, and 22-50 eggs dy^{-1} for *N.sp.* 98 was rounded up to 100, and 50 taken as the upper limit of fecundity, purely for the reasoning that these whole numbers allow easier examination of development through the early stages of the GIN life cycle.

The estimated life spans of adults of each of the studied genera (Table 5) is likely to be varied and is largely unknown for the less understood species. To avoid unnecessary error and reduce the number of unnecessary variables, all adult worms were considered to begin depositing eggs at their fixed fecundity rate on January 1st of the year, ceasing transmission on December 31st of the same year and with a different theoretical female then beginning deposition in the following year. To avoid the mixing of eggs and larval stages from different females, all remaining eggs and larvae at the end of their first year in the environment were separated into a separate series of identical model columns, to continue development and mortality over a 2nd and 3rd year to the point of depletion. No further eggs enter the model during the 2nd and 3rd years.

Table 5 Parameterisation - Adult female fecundity by genus.

Haemochus	5500	Eggs dy-1 per adult	Coyne & Smith (1992)
Nematodirus	50		Morgan et al. (2005)
Marshallagia	100		Morgan et al. (2005)

2.2.5 Development Rates

Full definitions of developmental stages and rate equations for each genus are shown in Table 6.

δE – Egg development to intermediate larval stages

While δE refers to development within the egg prior to hatching, this definition has differences for each of the 3 genera. In *Haemonchus* δE represents development to L1, in *Marshallagia* to L2 and in *Nematodirus* to L3.

Haemonchus development rate is a constant function of temperature. Rate equations were calculated by *Rose et al.* (2015). In this study, a series of lab-grown cohorts of *H.contortus* were allowed to develop at a range of fixed temperatures. The time taken at each stage to reach 50% development at each temperature were recorded and plotted to form a linear regression equation. δ in this study covers the entire life cycle from egg to L3; and is therefore separated and doubled to form a value of δE and δl.

Significance of desiccation on *Haemonchus* is recognised and included by preventing development of any surviving L3 to pasture in absence of adequate precipitation. Significant decreases in developmental success occur where evapotranspiration is greater than precipitation in the 24-96 hours following egg deposition (O'Connor *et al.* 2008, Kadijah *et al.* 2013). In this model, development will not occur at a temperature dependent rate unless cumulative precipitation/evapotranspiration remains greater than 1 for 4 days prior to any particular day within the model structure.

N.battus has a high minimum temperature for development and therefore a smaller window for development than *N.filicollis,* development of the former is also slower than the latter (van Dijk & Morgan 2009). Desiccation experiments showed that while extreme water removal using high osmotic pressures did kill eggs, overall development could persist across all early stages of the life cycle due to the protection

of larva within the egg (van Dijk & Morgan (2012). Rate equations are produced from these studies through the same method described under δh parameterisation.

Morgan (2003) evaluated Russian literature regarding *Marshallagia* development, deciding fixed rates of development at 5^{o}c temperature intervals. Sufficient studies were unavailable for the reliable generation of a linear development equation as in Rose *et al.* (2015). These 5^{0}c intervals form the development rates for *Marshallagia* in this study. Like *Nematodirus, Marshallagia* is resistant to desiccation within the egg.

δh – Hatching of intermediate stage larvae to free stage larvae.

This rate exists solely for *Marshallagia,* as in the case of *Haemonchus* and *Nematodirus* egg hatching is incorporated into δL.

Morgan (2003) noted that studies on the family Ostertagii such as Young *et al.* (1980) show a consistent hatching requirement of 14^{o}c, and that hatching takes place within 1 day of this threshold being met, assuming development of the L1 within the egg. This is the requirement for hatching used in this model.

δL – Intermediate stage larvae development rate to third stage infective larvae (L3)

For *Haemonchus,* like δE, the in-depth rate equation devised in Rose *et al.* (2015, 2016) has simply been doubled to allow separation of the initial life history stages. In *Marshallagia* this represents the development of hatched L2 to infective L3. In *Nematodirus* this represents solely the hatching of third stage larvae within the egg, becoming free infective L3. The exact same rate of development due to temperature, and moisture limitations on development, are applied to this portion of the model as in δE.

The time taken for 50% of embryonated larvae of *N.battus* and *N.filicollis* to hatch at fixed temperatures were recorded in Van Dijk & Morgan (2008) and (2009) respectively. Rates at these temperatures were calculated from the above data in Microsoft Excel by the method:

Rate = 1/d50

Rates at fixed temperatures were then averaged appropriately to produce rate equations at 5ºc intervals similar to that done in (Morgan *et al.* 2005).

Also involved in this model is a function for *Nematodirus* bet-hedging. Multiple studies have attempted to generate consensus on chilling requirements for development in species that exhibit bet-hedging, often the results differ significantly between studies. Studies on development of *N.battus* with or without chilling show that not all eggs hatch unless exposed to temperatures below 10ºc, with a further increased proportion of eggs hatching successfully with an exposure of temperatures at maximum 4ºc for 4 weeks (van Dijk & Morgan 2008).

In *N.filicollis* most recent studies have separated cohorts, with some eggs entering chilling for a fixed time period, while others are maintained at higher temperatures. Following this period eggs from both cohorts were allowed to develop at the same temperature for another fixed period. The proportion of individuals that hatch in each cohort was then recorded. Oliver *et al.* (2016) noted that hatching of New Zealand *N.filicollis* increased with increased chilling in a curvilinear relationship with an asymptote of 15% egg hatch, in comparison to Van Dijk & Morgan (2009) where 75% of UK *N.filicollis* eggs hatched following a return to 20ºc after 33 days at 4ºc.

The authors of these studies note the variation between studies historically: 35% of eggs hatched after 6 months at 3ºc in Thomas & Stevens (1960), and only 10% of developed eggs hatched after 11 weeks at 9ºc in Christie (1962). In both historical studies there was no return to warmer temperatures following the chilling period, there is doubt as to whether the differences are due to incompatible methodology or a difference in life history between geographical isolates (Oliver *et al.* 2016).

The development of *Marshallagia* from free L2 to infective L3 is rapid under the ideal conditions above 14ºc, particularly when temperatures reach above 20ºc. However, the success of *Marshallagia* in arctic climates suggests this may not be the norm across the whole range of this species (Carlsson *et al.* 2012). Until quantitative data

across this range is further understood, the parameters originally used to model development rate of Marshallagia (Morgan *et al.* 2005, 2007) are used here also.

Table 6 Parameterisation – Rates of environmentally dependent GIN development from freshly laid egg to L3, in the external environment used in the model.

Symbol	Parameter	Genus	Value	Source
δ	Daily development rate from egg to L3	Hae	−0.09746 + 0.01063(T)	Rose *et al.* (2015)
δ_E	Daily development rate from egg to intermediate larval stage	Hae	(-0.09746 + 0.01063(T))*2	Rose *et al.* (2015)
		Nem	<10°c = 0 >10°c, <15°c = 0.0206 >15°c, <20°c = 0.0273 >20°c, <25°c = 0.0355 >25°c, <30°c = 0.0379 >30°c = 0	Van Dijk & Morgan (2008, 2009)
		Mar	5°c = 0 10°c = 0.014 15°c = 0.028 20°c = 0.037 25°c = 0.044 30°c = 0.044	Morgan (2003)
δ_h	Daily egg hatch rate (if not incorporated into δ_E or δ_L)	Mar	If T <14°c = 0 >14°c = 1	Morgan *et al.* (2005)

δ_L	Daily development rate from intermediate larval stage to infective larval stage (L3)	Hae	(-0.09746 + 0.01063(T))*2	Rose *et al.* (2015)
		Nem	<0^{o}c = 0 >0^{o}c, <5^{o}c = 0.0263 >10^{o}c, <15^{o}c = 0.146 >10^{o}c, <15^{o}c = 0.334 >15^{o}c, <20^{o}c = 0.161 >20^{o}c = 0	Van Dijk & Morgan (2008, 2009)
		Mar	<15^{o}c = 0 15^{o}c = .17 20^{o}c = .25 25^{o}c = .5 30^{o}c = .5	Morgan (2003)

2.2.6 Mortality Rates

Mortality rate definitions and values are shown in Table 7.

Haemonchus mortality under varying temperatures is as equally well studied as development. Rate equations differ from those of development in that mortality rates are highest at extremes of temperature both hot and cold, therefore polynomial rate equations are used rather than linear equations. Again, in Rose *et al.* (2015), laboratory-based experiments recorded time taken to reach 50% mortality at each stage of the life cycle, forming a series of polynomial equations from the results of that study and those of similar efforts. *Haemonchus* mortality increases greatly under temperature extremes, more so than in the other 2 genera. The rainfall limitation applied to development combined with a high background mortality rate means an additional rainfall application in the mortality compartment is redundant.

N.sp eggs and embryonated larvae are so resistant to high temperatures and desiccation that it's extremely difficult to calculate exact mortality rates in lab

conditions. Some studies imply a background mortality rate of *Nematodirus spp.* close to zero, with embryonated eggs potentially surviving for 3+ years. In Morgan *et al.* (2005) *Nematodirus* mortality rates were estimated from their observed maximum persistence in the field, with the inclusion of some assumptions. Due to the lack of further available literature on mortality of relevant species, these rates are used in this model.

Nematodirus eggs and embryonated larvae are extremely resistant to drought. A period of 15 weeks at 33% humidity produced no significant mortality in dried *Nematodirus* eggs (Parkin 1976). In the case of infective stage larvae, records show *N.spathiger* was shown to be able to survive 166 days with no water at 15-20^{o}c and remain infective after this period (Van Dijk& Morgan 2012). Therefore, no inclusion of precipitation or evapotranspiration is included in mortality rate calculations for this genus.

M.marshalli suffers a far higher mortality rate under conditions suitable for development at the later stages of the external life cycle than earlier stages, which may explain the trending of the species towards rapid development from L2 to infective L3 after hatching (Morgan 2003). Rates are calculated from an assortment of historic literature in (Morgan 2003), including some from arctic studies due to the lack of better data. These rates are used in this model.

Table 7 Parameterisation - Rates of environmentally dependent GIN mortality throughout the free-living larval stages of the GIN life cycle used in the model.

Symbol	Parameter	Species (If Varied Between)	Value	Source
μ_E	Daily mortality rate of eggs	Hae	$\exp(-1.62026-0.17771T+0.00629T^2)$	Rose *et al.* (2015)
		Nem	<10^{o}c = 0.003 >10^{o}c = 0.006	Morgan (2003)
		Mar	<-15^{o}c = .024	

			<-10^{o}c = .012 >0^{o}c = .01 >15^{o}c & Prec 10dy >10mm = .01 >15^{o}c & Prec 10dy <10mm = .12	Morgan *et al.* (2005, 2007)
μ_L	Daily mortality rate of intermediate larval stages	Hae Nem Mar	exp(−1.82300−0.14180T+0.00405T^2) <10^{o}c = 0.003 >10^{o}c = 0.006 <-15^{o}c = 1 <-10^{o}c = .23 >0^{o}c = .01 >15^{o}c & Prec 10dy >10mm = .01 >15^{o}c & Prec 10dy <10mm = .23	Rose *et al.* (2015) Morgan (2003) Morgan *et al.* (2005, 2007)
μ_{L3}	Daily mortality rate of infective stage larvae	Hae Nem Mar	exp(−2.63080−0.14407T+0.00463T^2) <0^{o}c = 0.00285 >0^{o}c, <10^{o}c = 0.0033 >10^{o}c, <15^{o}c = 0.00378 >15^{o}c, <20^{o}c = 0.00212 >20^{o}c, <25^{o}c = 0.0325 >20^{o}c, <30^{o}c = 0.0827 >30^{o}c = 0.1195 <-15^{o}c = .5 <-10^{o}c = .024 >0^{o}c = .01 >15^{o}c & Prec 10dy >10mm = .01 >15^{o}c & Prec 10dy <10mm = .012	Rose *et al.* (2015) Morgan (2003) Morgan *et al.* (2005, 2007)

2.2.7 Movement to Pasture and Survival on Pasture

Rate equations and definitions are shown in Table 8

m1 – Transition rate of stage 3 larvae from soil to pasture.

Experiments in Rose *et al.* (2015) on lab grown *Haemonchus contortus* and *Teladorsagia circumcincta* showed that simulating daily precipitation of a minimum of 2mm provided ideal conditions for migration of infective L3 to pasture. Migration was still possible at a reduced rate below this level of precipitation, as long as evapotranspiration is not higher than precipitation over the 4-day period prior to the attempted migration.

The requirement for a free infective nematode larva belonging to any genus to migrate to pasture is likely to be similar between species. Therefore, it is appropriate to apply the same rate and limitations across all 3 genera (Morgan, personal communication),

mL3p – Mortality rate of stage 3 larvae on pasture.

Haemonchus mortality remains relatively high due to increased pasture exposure, although the species is still more resilient as infective L3 than free L1/L2. (Rose *et al.* 2015)

N.sp mortality on pasture is approximately similar to that of free infective L3 in soil due to the resistance of this genus to increased exposure (Van Dijk & Morgan 2012).

Marshallagia mortality rate was doubled in (Morgan *et al.* 2005) to replicate the increased exposure on herbage; this value is used in this model.

Table 8 Parameterisation – Environmentally dependent movement to pasture and survival on pasture rate equations used in the model.

Symbol	Parameter	Species (If Varied Between)	Value	Source
μ_{L3p}	Daily mortality	Hae	$\exp(-2.63080-0.14407T+0.00463T^2)$	Rose *et al.* (2016)

	rate of infective stage larvae on pasture	Nem Mar	<10°c = 0.008 >10°c = 0.016 <-15°c = .5 <-10°c = .024 >0°c = .01 >15°c & Prec 10dy >10mm = .01 >15°c & Prec 10dy <10mm = .024	Morgan *et al.* (2005)
m_1	Migration rate of infective larvae from faeces to pasture		If P≥2, 0.25 If P<2, and P/ET0>1 for 4 days prior, 0.051 If P<2, and P/ET0<1 for 4 days prior, 0	Rose *et al.* (2016)

2.2.8 Obtaining host presence data

Saiga numbers have been estimated throughout the Soviet era and into the 21st century using aerial counts and physical monitoring by running transects through Spring calving aggregations and counting calves (Table 9). In the model, host population size is considered constant from January 1st to December 31st each year. Despite the population increase in Spring, mortality will be distributed unevenly across the entire year, so tying the interannual change in population size to the Spring months would be unsuitable.

Table 9 Estimated saiga antelope population size from 1979 to 2017.

Year	Betpak-Dala Saiga Population Size	Source
1979	290000	Bekenov *et al.* (1998)
1980	400000	
1981	470000	

1982	480000	
1983	440000	
1984	340000	
1985	400000	
1986	250000	
1987	300000	
1988	368000	
1989	323000	
1990	261000	
1991	357000	
1992	375000	
1993	510000	
1994	282000	
1995	212000	
1996	248000	
1997	184000	Milner-Gulland *et al.* (2001)
1998	120000	
1999	64000	
2000	15000	
2001	12000	Singh *et al.* (2011) Dr Sarah Robinson (personal communication)
2002	4000	
2003	1800	
2004	6900	
2005	9900	
2006	16800	
2007	22800	
2008	32300	
2009	45200	
2010	53400	
2011	78000	
2012	110000	
2013	155200	

2014	216700	
2015	242500	
2016	36200	
2017	36200	

2.2.9 Calculating intake of pasture L3 by hosts

Rate of intake of pasture L3 by hosts is defined as:

c/B*Area

Where c is daily herbage dry matter intake per host and B is biomass (KG DM ha^{-1} for both variables) (Rose *et al.* 2015).

Average values of Betpak-Dala biomass and saiga vegetation ingestion rate were obtained from Robinson (2000). Biomass does vary with climate, and models exist to account for this. Time constraints and the goals of the model led to this being absent in this study, but it is recommended that such a compartment be added in future modelling efforts in this style.

Host area ranges were calculated from the approximate ranges shown in Singh et al. (2011) mapped over software to extract surface area. Some range size averages are included in Bekenov *et al.* (1998), including the average size of a calving aggregation. Average saiga host densities are available, but the purpose of this model is to determine how changes in host population size may be significant for transmission in this scenario, therefore host density must vary over time.

An adjustment factor of 0.4 is added at the final step to account for the probability of ingested L3 establishment in the host (Rose *et al.* 2016).

Full rate definitions and values are shown in Table 10.

Table 10 Parameterisation - Demographical variables and ingestion rate calculations used in the model.

Symbol	Parameter	Region	Value	Unit	Source
c	Daily Herbage Dry Matter Intake per host		1.4	Kg DM ha^{-1}	Robinson (2000)
B	Biomass		862	Kg DM ha^{-1}	Robinson (2000)
Area	Host Area Range	North Centre 1.0 Centre 2.0 South	18000000 37500 28000000 6000000	ha (converted from km^2)	Bekenov *et al.* (1998). Singh et al. (2011)
H	Host Density	North Centre South	n/AreaNorth n/AreaCentre n/AreaSouth	ha^{-1}	
β	Rate of ingestion of L3p		c/B*Area		Rose *et al.* (2016)
ρ	Probability of establishment of ingested L3		0.4		Rose *et al.* (2015)

2.3 Usage of the model – Scenarios

The model is applied over 2 basic scenarios that revolve around host density during the Saiga range. 2 additional scenarios are then separately applied to both_basic scenarios for a total of 6 scenarios. These are named saiga aggregation (1.0), saiga aggregation with constant host density (1.1), saiga aggregation with livestock inclusion (1.2), no saiga aggregation (2.0), no saiga aggregation, with constant host density (2.1) and no saiga aggregation, with livestock inclusion (2.2).
Climatic conditions remained identical across all 6 scenarios.

Scenarios 1.0 and 2.0 differ primarily over the Saiga range size during the spring and autumn aggregations. During these aggregations host density is much higher than in the northern and southern ranges, which would result in increased probability of parasite transmission. For the genera studied in this thesis with longer external life-cycles, eggs and larval stages may survive for 2+ years in the external environment; during this time their location does not change.

In the northern and southern ranges, all individuals are considered equally distributed across the area, exactly like their hosts. To apply this rule to the mid-range would require saiga return to the same calving spot in each year, which in real life is unlikely (Singh *et al.* 2010, 2011). The creation of 2 base scenarios allows an estimation of parasite transmission under the assumption that saiga aggregations do return to the same spot annually, and an estimation that they do not; in which case individuals are evenly distributed over their maximum range.

Additionally, scenario 1.0 accounts for the reduction in Saiga summer and winter range sizes observed with the recent reductions in population size (Singh *et al.* 2011). This is not included in scenario 2.0 to maintain the testing of transmission potential over reduced host densities.

1.0 Saiga aggregation - Saiga are assumed to aggregate in the same location every year, and all individuals in the population are assumed to share this space. Host range size during the spring and autumn aggregations is 37500 hectares. When Saiga range size falls below 100,000 individuals, the range sizes of the northern and southern

ranges are halved from 18,000,000 hectares to 9,000,000 hectares and 6,000,000 hectares to 3,000,000 hectares respectively.

2.0 – No saiga aggregation - Just as in the northern and southern ranges, Saiga are assumed to distribute across their Spring range equidistantly, host density is calculated by dividing saiga population size by the size of the range of Spring calving aggregations: 28,000,000 hectares. Northern and southern ranges are not reduced when population size drops, remaining at maximum.

1.1/2.1 – Saiga aggregation and constant host population size /No saiga aggregation, and constant host population size - Partial removal of host density as a factor. To attempt to disentangle the factors of host density and climate affecting the different stages of transmission, the current historical range of saiga population sizes was removed from the model and replaced with a constant host population size of 300,000 individuals. Unlike scenarios 1.2/2.2, these 300,000 saiga are still only present in each region only during the normal timing of Saiga within each region. Variation in transmission success between years in these scenarios can be considered solely due to variation in climatic factors.

1.2/2.2 – Saiga aggregation and livestock inclusion / No saiga aggregation, and livestock inclusion Non-zero values of hosts were used when Saiga are not present, to study the effects of reservoir populations on ability of the chosen parasite species to survive, and in turn make some conclusions about what effect changes in migration pattern might have.

Typically, in livestock-parasite systems, livestock are kept within a certain region and therefore total parasite population is concentrated over a much smaller area than in the Saiga system (Morgan *et al.* 2007). The implications for Saiga parasite acquisition are large if Saiga spend any period of time in a space occupied by livestock throughout the year.

As this model considers a female depositing from a single Saiga over the course of the year, it is impossible to model whether or not transmission from livestock back to Saiga is possible, as in Morgan *et al.* (2007). Instead, while normally when the Saiga

leave the compartment of the model belonging to North/Centre/South range, host density drops to zero, instead a minimum host density of 0.1 hosts per hectare remains in all locations at all times of year. What this allows is for individuals that pass through the model to pasture L3p to simply decline at L3p mortality rate until the Saiga return, to instead be ingested by the “livestock” host at the appropriate ingestion rate.

Effectively, this provides an answer to the question, “is total R_0 of a single adult female GIN increased by the presence of livestock across the saiga population range?”, and by extension “is it likely that the setting up of reservoir populations could maintain a population of a particular GIN species when Saiga alone are in too low numbers to maintain adequate transmission?”

Nematodirus chilling factor

As mentioned previously throughout parameterisation, the involvement of bet-hedging can have great effects on the success of *Nematodirus* genera. While *Haemonchus* and *Marshallagia* each have 1 model, *Nematodirus* has been split into 2: *Nematodirus* chill (NC) and *Nematodirus* no chill (NNC). The models are parameterised equally with the exception that hatching of the egg in NC requires the average daily temperature of the 28 days preceding the day in question to be below 4^{o}c. When this is achieved, all fully developed in egg larvae are considered “active” and begin to be transferred to the “hatched L3” column at the appropriate daily rate.

In NNC, this delay is not present, and any fully developed larvae in eggs may develop to hatched L3 immediately should conditions be viable. In reality, only a proportion of offspring produced by a single female will delay hatching, and this proportion varies within and between *Nematodirus* species. Therefore, this modelling effort opts to study the effects of 2 extremes, where in NC 100% of eggs require chilling to hatch, and in NNC 0% of eggs require this chilling. Each of the 6 scenarios is applied to both *Nematodirus* model structures.

2.4 Model Validation

2.4.1 Statistical Records

Precise model validation is difficult for a model that itself lacks a focus on precision. The outputs of this model are interpreted primarily as a binary “can or cannot” theoretical individual parasite genera persist under the circumstances of each modelled scenario.

This is compared against similar presence/absence data taken from historical Soviet era literature, field studies in Kazakhstan during the late 1990’s and early 2000’s, and the results of a fieldwork expedition carried out as part of this study during 2017. The methodology regarding this field study is written below.

2.4.2 Fieldwork (subsection of model validation)

To support the climate and parasite data available online and in scientific literature, more recent field data was acquired from Kazakhstan. From May 6th-20th 2017 3 members of Bristol University Veterinary Parasitology, myself included, joined an Association for the Conservation of Biodiversity of Kazakhstan (ACBK) led expedition, primarily aimed at surveying Betpak-dala Saiga calving grounds during the spring migratory period. A 4th member carried out parallel data collection on a separate ACBK expedition in the Ural region. The majority of sample collection took place in the steppe during the expedition. When time limited the ability to process all collected samples on a particular day, specimens were preserved and processed in an improvised laboratory at ACBK headquarters in Astana, Kazakhstan, between May 22nd-26th 2017.

<u>Climate Data</u>

To survey the climate during the expedition Maxim iButton® DS1923 temperature/humidity loggers were used. The device has shape and size similar to a button cell battery. An individual device records 8-bit readings of temperature and humidity at equidistant intervals of 10 minutes, continuously for the duration of the expedition. At each expedition encampment, 2 button cells were placed at ground level to record for the duration and were retrieved immediately before the expedition

continued to the next site. The time of placing and collection of iButtons was recorded in order to extract the appropriate recordings from the complete dataset upon return to the UK. Temperature recordings are accurate to ±0.5^{o}c, humidity recordings are accurate to 0.6%RH (maximintegrated.com).

Helminth Data

To examine the helminth population in Kazakhstan, Saiga faecal samples (n=117) were collected whenever possible during the expedition, most commonly while carrying out 5km transects within a calving area, where fresh faeces are most abundant. With permission from farmers in the region we were also able to collect faecal samples from ruminant livestock. A GPS device was used to record the exact location that each faecal sample was obtained. As helminth eggs of different species vary in their ability to survive in desiccated faeces, only fresh faecal matter was collected to ensure no bias in sampling. Freshness of faeces was determined by eye/touch, Saiga pellets lose moisture within hours of dropping, becoming lighter in colour and non-malleable. Collected faecal samples were placed in sealable plastic bags or latex gloves until processing at camp during the evening.

The fill-FLOTAC method was employed to carry out all faecal egg counts (FEC). For each individual faecal sample, 5g of faecal matter was taken and homogenised with 45ml of saturated NaCl flotation solution in the fill-FLOTAC. Using a hydrometer, salt solution was prepared in the field to specific gravity 1.20. In the rare event that the faecal sample contained less than 5g of matter, the entire sample was homogenised with 9ml of flotation solution for each gram of matter. Following inversion to ensure thorough mixing, 1ml of the mixture was added to each of the chambers of the FLOTAC disk. After a minimum of 10 minutes rest period, number of eggs present in the contents of the FLOTAC disk were recorded using a battery-powered light microscope at 40x or 100x magnification and multiplied by 5 to produce an eggs per gram (EPG) measurement.

If all faecal samples could not be processed on the day of recovery, they were preserved in 5% formalin solution at a minimum ratio of 1:4, 1-part faecal matter to 4 parts preservative as outlined in Cringoli *et al.* (2010). Preserved samples were

processed when possible in the field or in Astana using the above FLOTAC method. Towards of the end of the expedition, extremely limited preservative availability required that improvised anaerobic preservation be used to preserve samples for 3-6 days before processing in Astana. Faecal samples were placed in marked tubes, tubes were then filled with water and sealed. Decay of material is limited by removing the oxygen supply to micro-organisms present within the tube.

Graticules present in light microscopes allowed measurement of a portion of collected eggs as time allowed. Microscope graticules were calibrated using the standard method of observing the number of divisions of a stage micrometer.

Freshly deceased adult saiga carcasses were discovered in both expeditions, 1 in Betpak-Dala and 2 in Ural. Full dissections of the carcasses were carried out by ACBK staff or researchers from the Royal Veterinary College, London. The abomasum was identified and isolated from each saiga carcass. Additionally, in Betpak-Dala a single ruminant offal of a recently slaughtered livestock animal was donated by a Kazakh farmer. In Betpak-dala the abomasa and livestock small intestine were washed with a small amount of water, all contents were collected and stored in marked tubes with 5% formalin preservative. In Ural, abomasal washes were not possible, instead small samples of abomasal contents were preserved in 5% formalin. Processing of samples took place in the Astana laboratory; gut contents were poured through a 212µm sieve to separate any mature worms from organic material. Filtered contents were transferred into a plastic container holding water. 50ml at a time of filtered contents were transferred into a separate container. From this 50ml, 5-10ml of mixture was placed onto a petri dish, adult worms in the petri dish were identified using a hand lens, counted and moved from the mixture into a marked tube denoting the carcass of origin. ~100 adult worms are typically required for a representative sample, however due to the low number of adults collected, this was only achieved from 1 of the 5 abomasa/gut contents.

Adult worms were replaced in 2ml of 5% formalin preservative in small, marked tubes and returned to the UK under a general import authorisation No, IMP/GEN/2014/06 as allowed by the European Communites Act 1972.

In a Bristol University Life Sciences laboratory, adult worms were transferred from preservative onto microscopes slides and stained with lactophenol as is common procedure for worm identification (Bybd *et al.*1983), Individuals were studied under a light microscope at various magnifications to attempt to identify species or genus presence using identifying characteristics Skrjabin *et al.* (1954) was used as reference material.

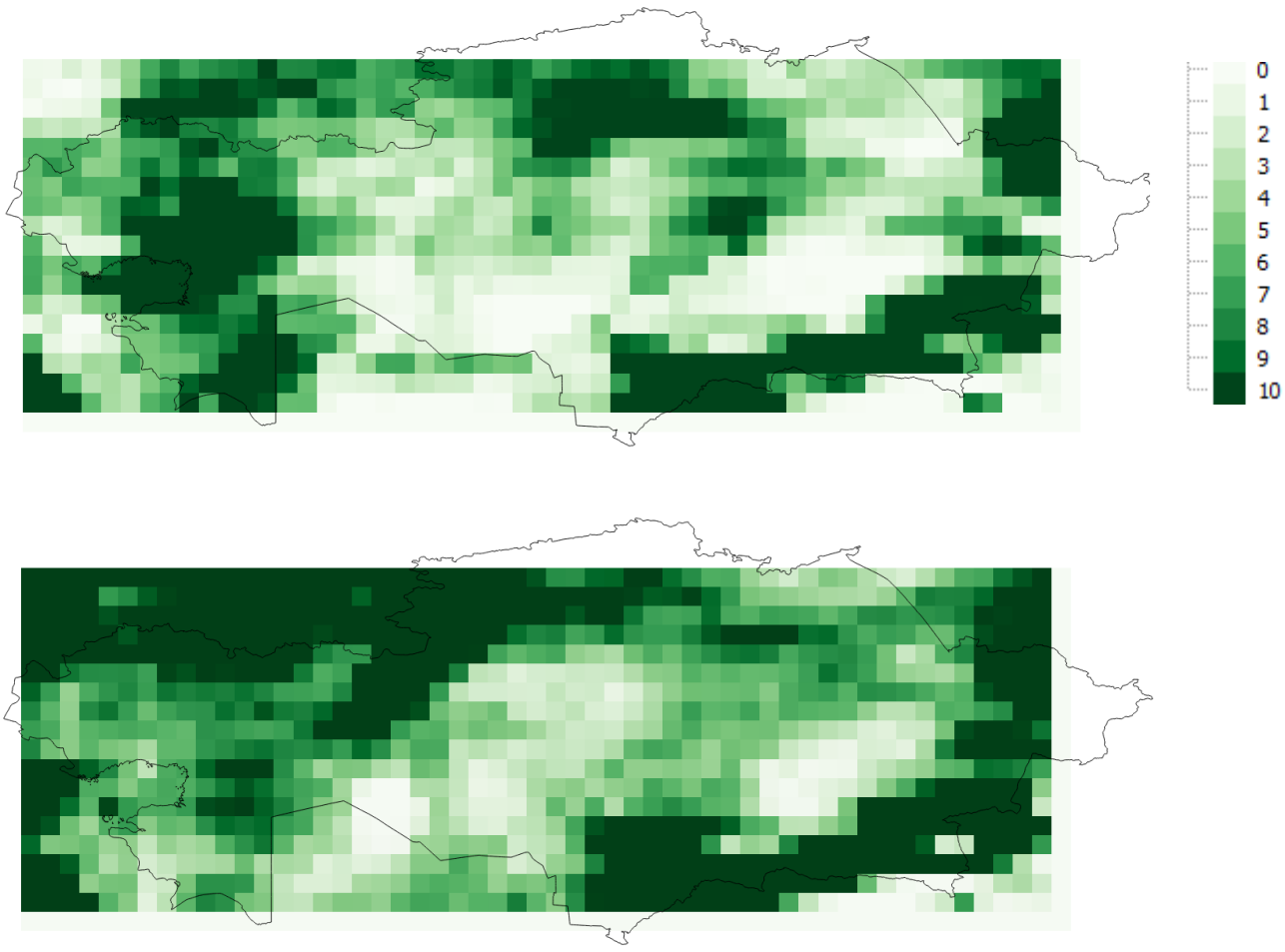
3.0 Results

This section includes some salvaged maps from early Q_0 model efforts in R, a series of model outputs from the R_0 model regarding the outlined scenarios applied to the parameters of each parasite genus, and lastly model validation data from historical literature and fieldwork.

3.1 Original Q_0 Model

Although some data from original Q_0 modelling efforts was lost, average *Haemonchus* daily Q_0 over the months of April-May, further averaged by decade from 1979-2016, was salvaged and included here (Figure 7).

Q0 is found to be low over the saiga range in most years, but significantly >1 in some regions. The conclusions we take from this in the context of the secondary model are limited however, due to the differences in moisture limitations used between models, and the inclusion of a fixed host stocking rate of 1 host per hectare.



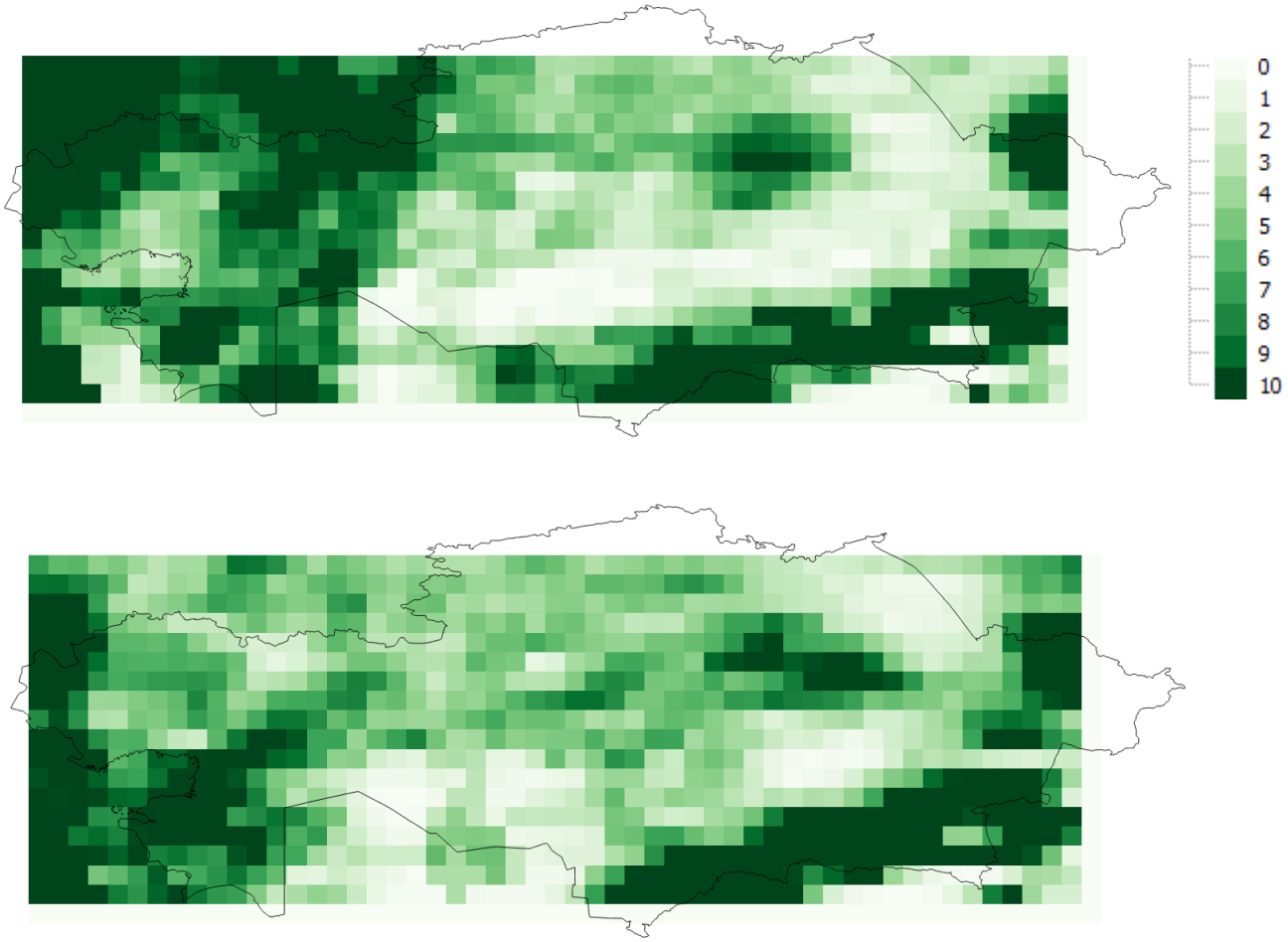


Figure 7) Maps of a basic Q_0 model for Haemonchus contortus over the region of Kazakhstan. Average daily Q0 during the months of April/May, averaged by decade. In descending order: 2009-2015, 1999-2008, 1989-1998, 1979-1988. Host density = 1, only climatic conditions are in effect. Each grid square represents a 1000x1000m area across the latitudinal and longitudinal range of the saiga distribution in Kazakhstan. Gradient in colour across grid-squares is in accordance with numerical estimates of Q_0 averaged over the above-mentioned time period, in each grid square.

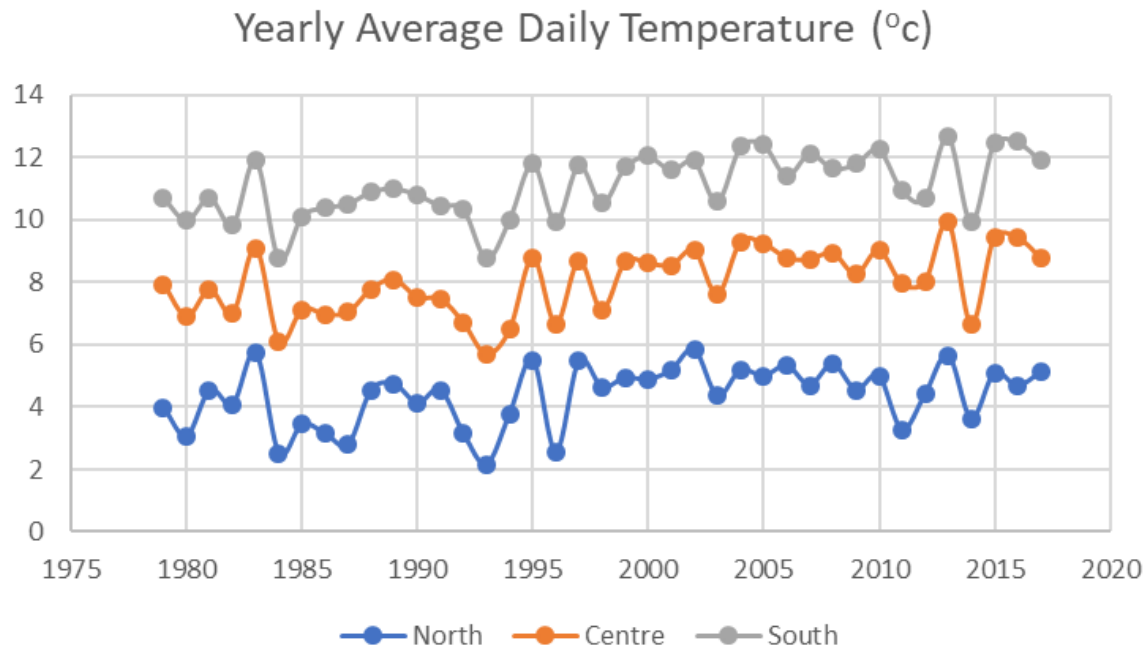
3.2 Excel Based R_0 model

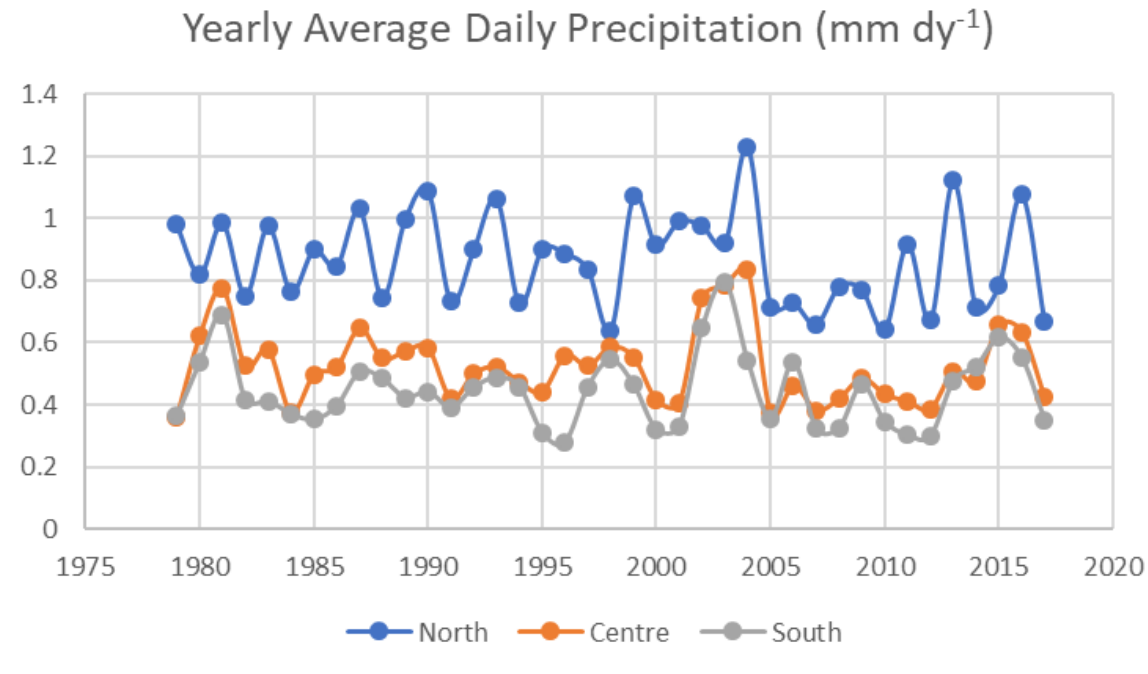
Climate Data

Temperature is significantly different between Saiga range areas on average of the course of this year, with North on average warmer than Centre and Centre on average warmer than South (Figure 8). Precipitation varies little between Centre and South ranges, but North is on average wetter within years. Over the temporal range of this

study, average temperature increases over time, average precipitation remains highly stochastic between years (Figure 8).

Figure 8) Yearly average climatic variables applied to each part of the Saiga range in the excel-based R0 model.

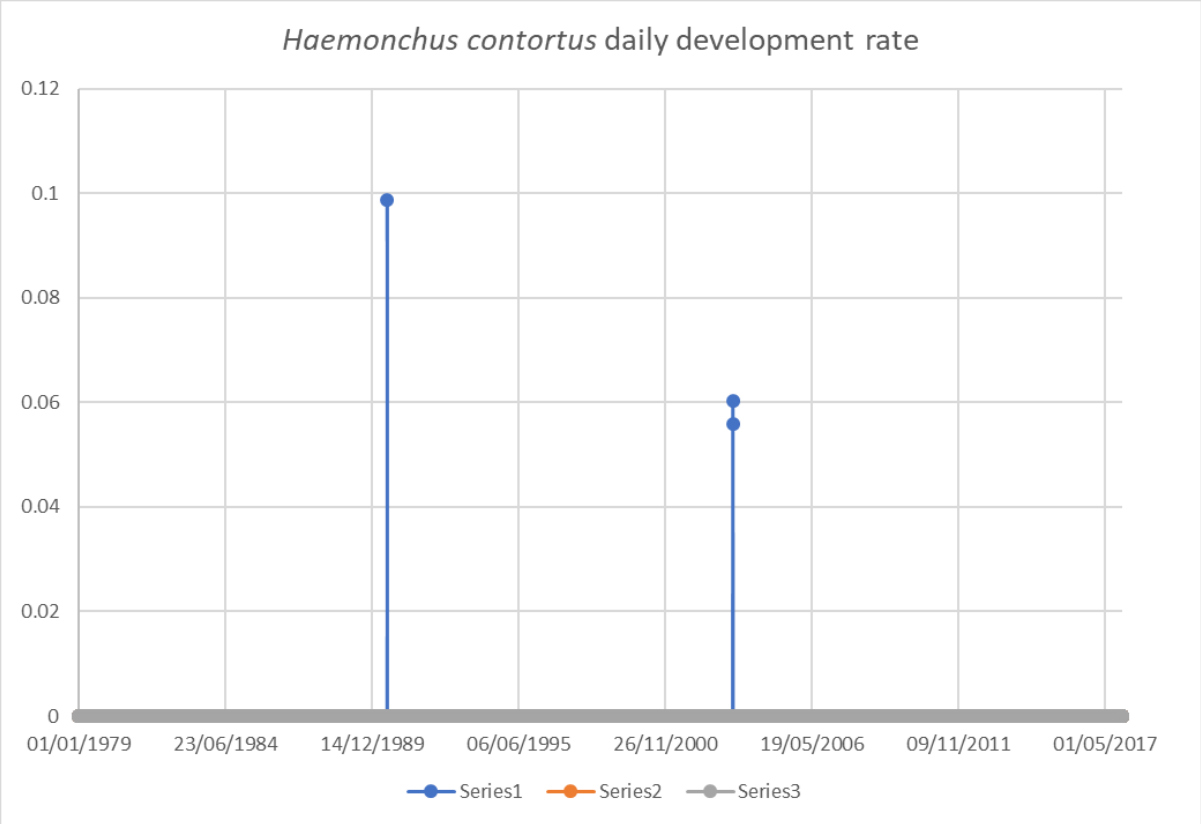




Haemonchus contortus

H.contortus failed to produce any value of R_0 larger than 0 at any time of year across the entire range of years, due to the moisture requirements of cumulative Precipitation/Evapotranspiration being positive four days prior to the day of rate application, only being met on a total of three days (***Figure 9***). *H.contortus* is therefore not included in graphs regarding transmission in each scenario in the following section.

Figure 9) H.contortus daily development rates δ, over yearly range. Series 1 = Northern Range, Series 2 = Centre Range, Series 3 = Southern Range. At no point across the time period was daily development rate >0 in the centre and southern ranges and therefore no points are visible.



Scenario Comparisons

The following graph series shows transmission of *Nematodirus* with chilling factor (NC), *Nematodirus* without chilling factor (NNC), and *Marshallagia* (M), under six scenarios. Log_{10} y-axis scale shows total R_0 of single female alive in x year.

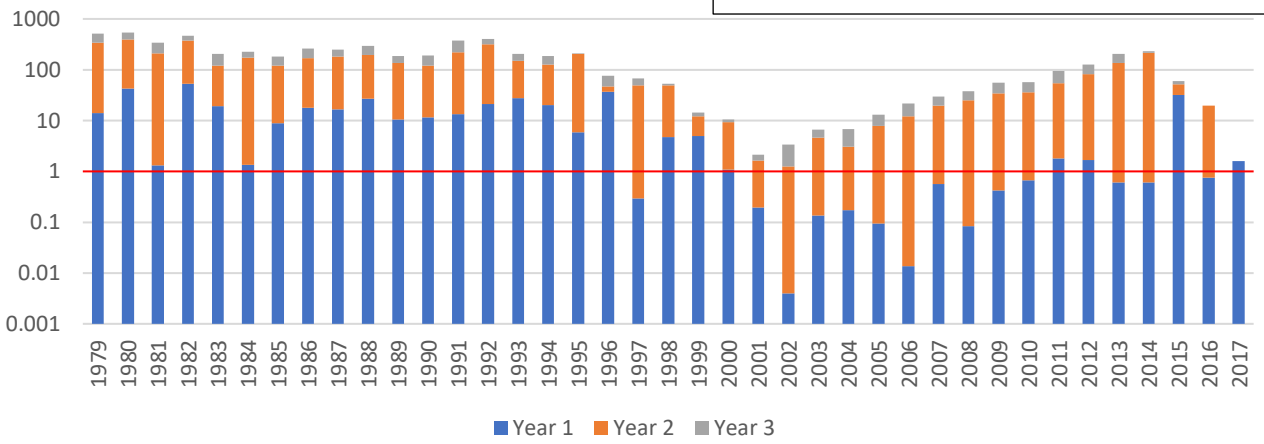
NC = *Nematodirus* with a chilling requirement for development

S1 = Saiga aggregation scenario

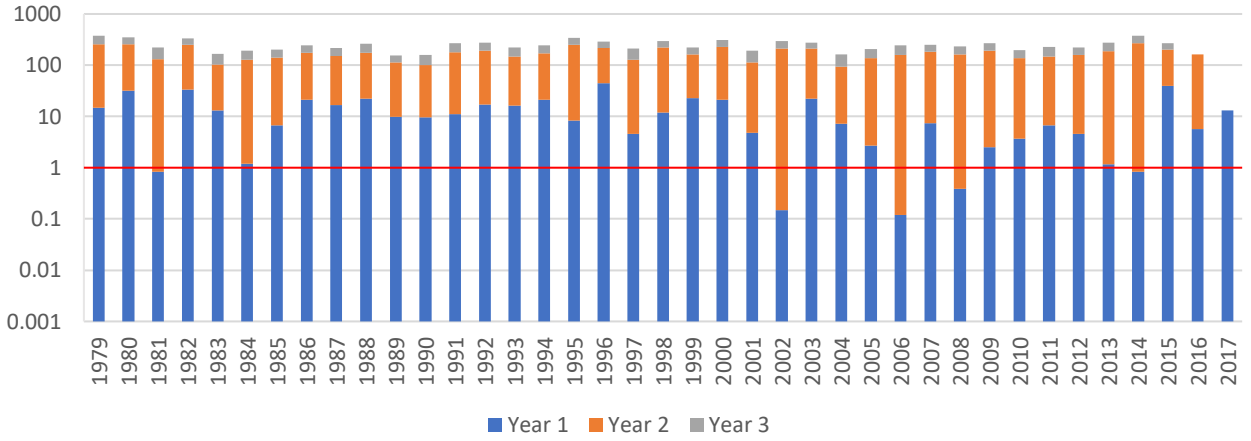
S1.1 = Saiga aggregation + constant host population size

S1.2 = Saiga aggregation + livestock inclusion

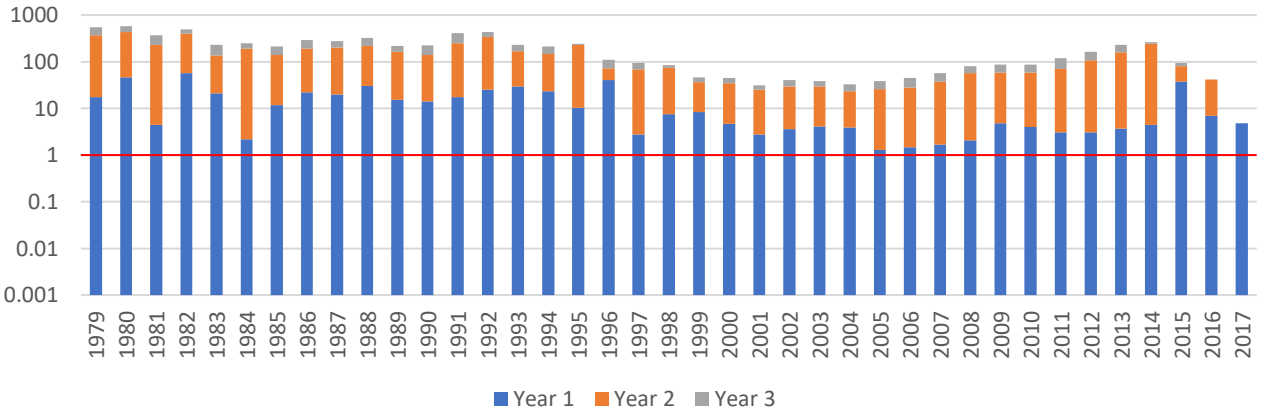
a) NC S1



b) NC S1.1



c) NC S1.2



NC = *Nematodirus* with a chilling requirement for development

S2 = No saiga aggregation scenario

S2.1 = No saiga aggregation + constant host population size

S2.2 = No saiga aggregation + livestock inclusion

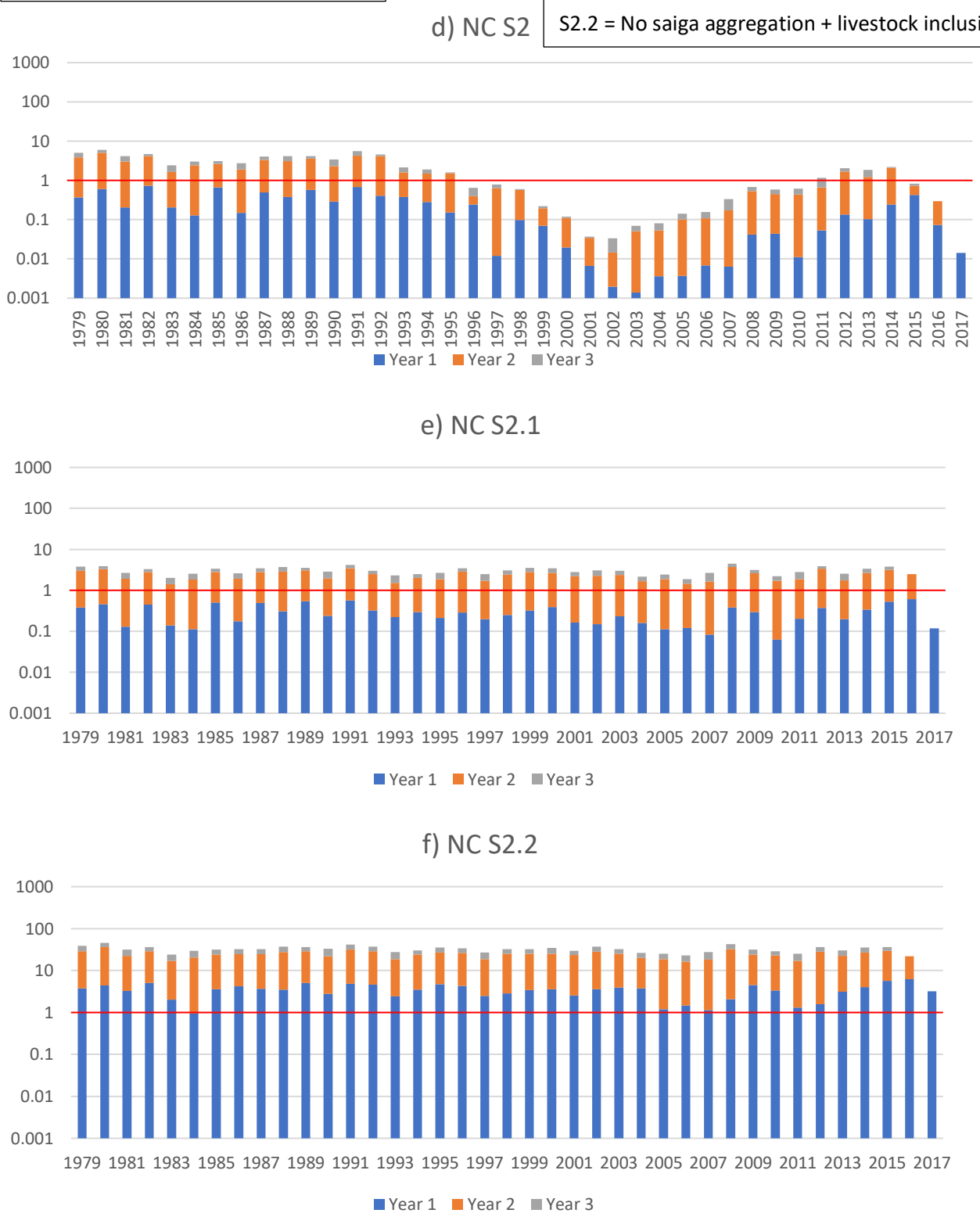


Figure 10) Series of graphs showing transmission (R_0) of Nematodirus with chilling function included. a) Spring aggregation and smaller northern and southern ranges included. b) including fixed population size. c) including livestock presence. d) no aggregation, maximum north and south range. e) including fixed population size. f) including livestock presence.

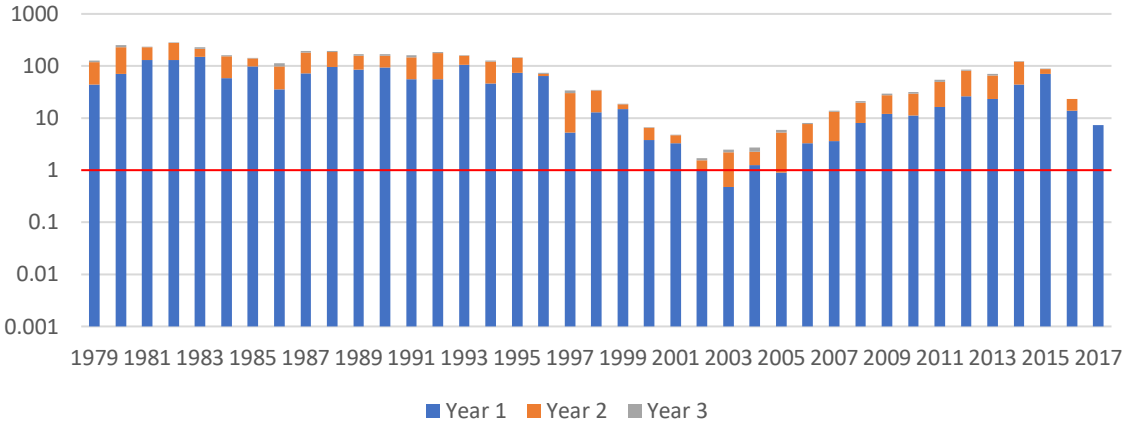
NC = *Nematodirus* with no chilling requirement for development

S1 = Saiga aggregation scenario

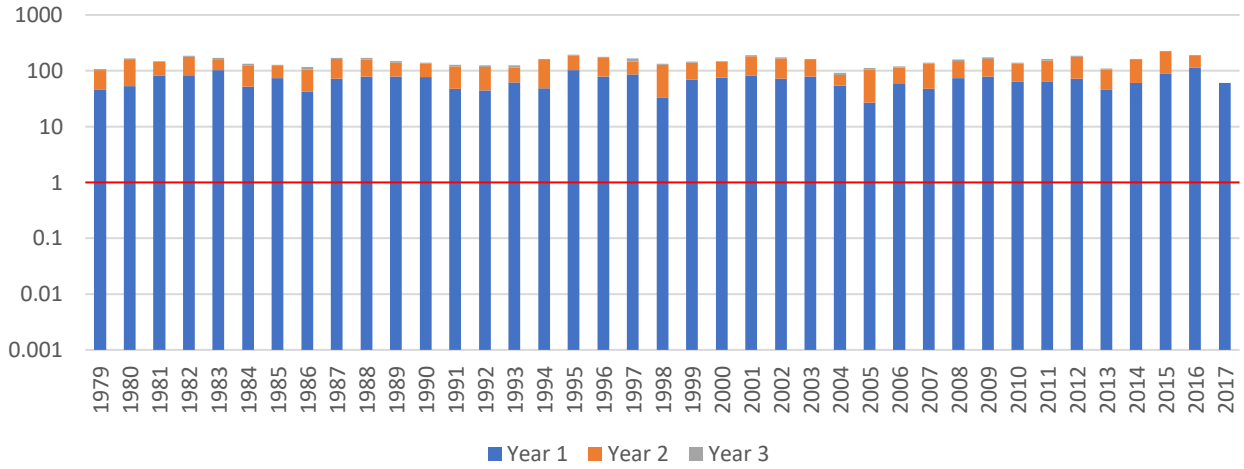
S1.1 = Saiga aggregation + constant host population size

S1.2 = Saiga aggregation + livestock inclusion

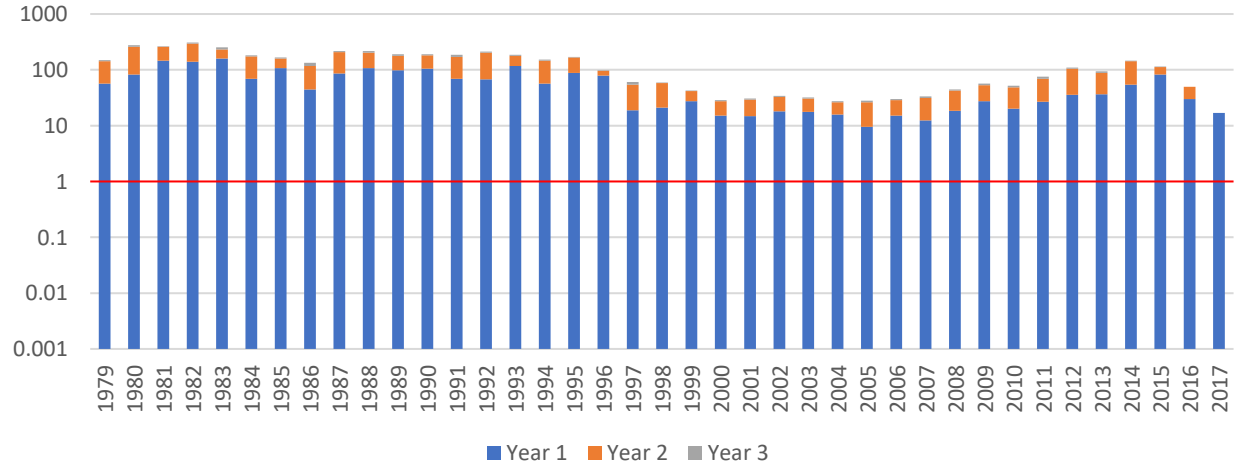
a) NNC S1



b) NNC S1.1



c) NNC S1.2



NC = *Nematodirus* with no chilling requirement for development

S2 = No saiga aggregation scenario

S2.1 = No saiga aggregation + constant host population size

S2.2 = No saiga aggregation + livestock inclusion

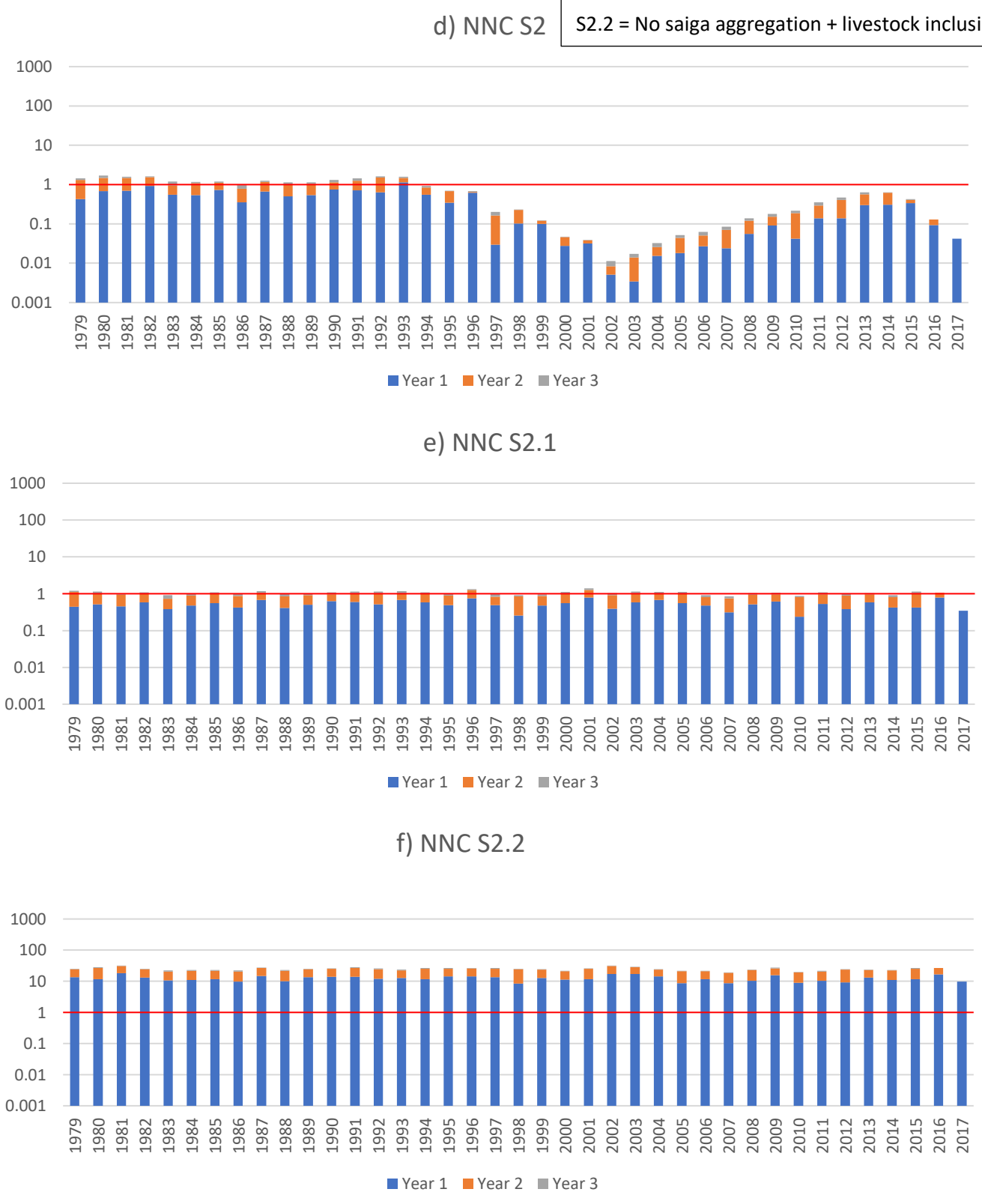


Figure 11 Series of graphs showing transmission (R_0) of Nematodirus with no chilling function included. a) Spring aggregation and smaller northern and southern ranges included. b) including fixed population size. c) including livestock presence. d) no aggregation, maximum north and south range. e) including fixed population size. f) including livestock presence.

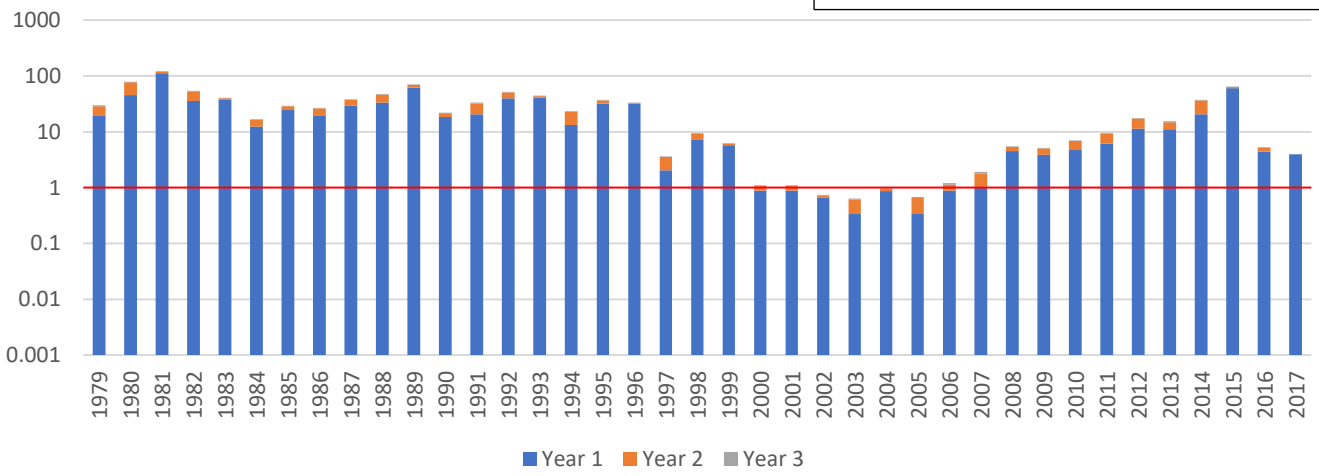
M = *Marshallagia*

S1 = Saiga aggregation scenario

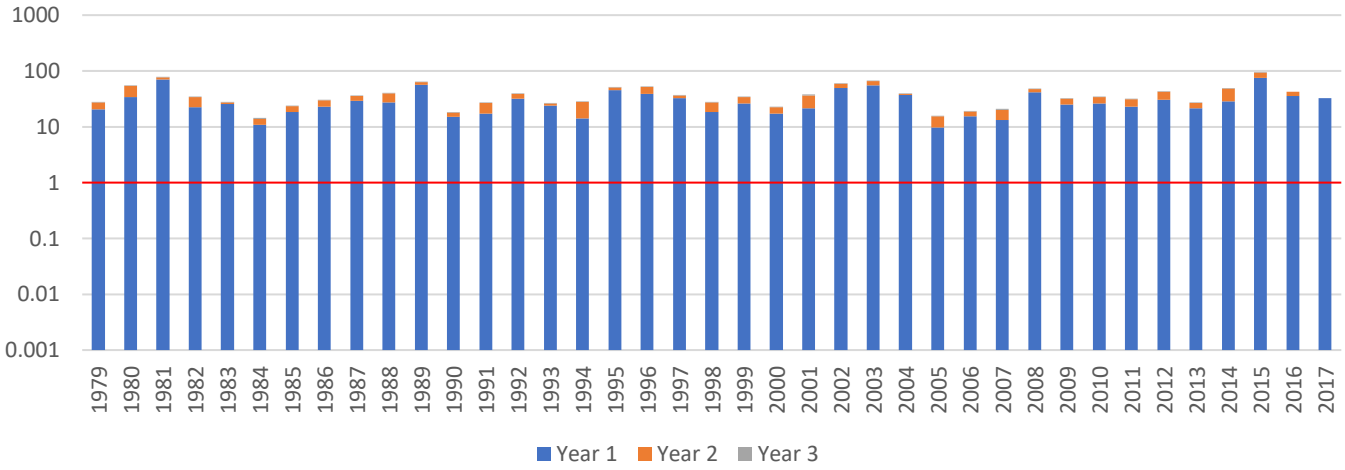
S1.1 = Saiga aggregation + constant host population size

S1.2 = Saiga aggregation + livestock inclusion

a) M S1



b) M S1.1



c) M S1.2

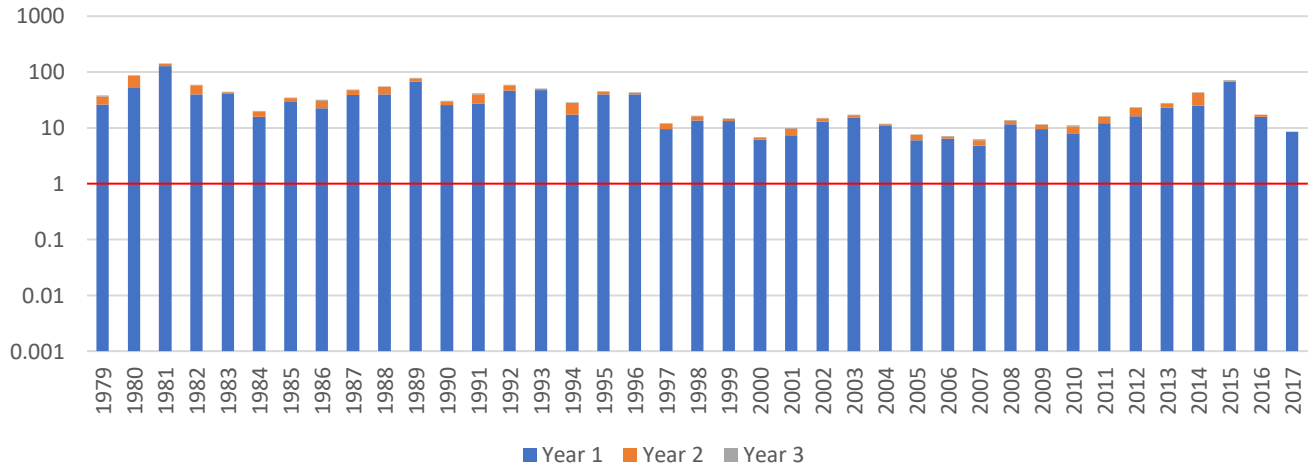


Figure 12 Series of graphs showing transmission (R_0) of Marshallagia. a) Spring aggregation and smaller northern and southern ranges included. b) including fixed population size. c) including livestock presence. d) no aggregation, maximum north and south range. e) including fixed population size. f) including livestock presence.

Scenario Series 1

1.0) Inclusion of Spring/Autumn aggregation and reduced northern and southern ranges when host population size falls below 100,000 individuals.

Nematodirus (NC) with a chilling requirement and *Nematodirus* with no chilling requirement (NNC) saw positive R_0 across all years (Figure 10a, 11a), with a reduction in total R_0 corresponding to dramatic decrease in host density during the 90's and early 2000's. In *Marshallagia* (M), R_0 dropped below 1 from 2002-2005, when host density was at its lowest (Figure 12a).

1.1) (1.0) with fixed host population size of 300,000 individuals across all years.

Nematodirus (NC) with a chilling requirement, *Nematodirus* with no chilling requirement (NNC) and *Marshallagia* (M) had positive R_0 across all years (Figure 10b, 11b, 12b). Slight fluctuation between years show that variation in climatic conditions is affecting transmission success in the absence of host density as an inter-annual variable.

1.2) (1.0) with inclusion of "livestock" at fixed density in all ranges at all times of year.

Nematodirus (NC) with a chilling requirement, *Nematodirus* with no chilling requirement (NNC) and M had positive R_0 across all years (Figure 10c, 11c, 12c). Compared to (1.0) yearly R_0 values were similar across all three genera, however the negative effects of the drop in saiga population size are significantly diminished.

Scenario Series 2

2.0) Spring/Autumn host distribution is even across maximum range size, no reduction to northern and southern ranges when host population size is reduced.

R_0 across all three remaining species parameters was significantly reduced in the absence of aggregation (Figure 10d, 11d, 12d). For *Nematodirus* (NC) with a chilling requirement and *Nematodirus* with no chilling requirement (NNC), transmission potential remains slightly positive until the collapse of Saiga populations from 1996 onwards. In *Marshallagia* (M), without the spring aggregation host density and a

reduced northern and southern range sizes, the model suggests that sustained transmission of this species will never occur. With a chilling factor, *Nematodirus* has positive R_0 from 2012 to 2014 as saiga population sizes return, although this is not the case in the model without the chilling factor. This is perhaps due to the delaying of hatching resulting in better synchrony of L3p with host presence.

2.1) (2.0) with fixed host population size of 300,000 individuals across all years.

Nematodirus with a chilling factor (NC) sees positive transmission success without the collapse in hosts. In all cases it is transmission during the second year after deposition that pushes R_0 over one, with the first year alone being inadequate (Figure 10e).

For *Nematodirus* without a chilling factor (NNC), R_0 is on the border of 1 across all years (Figure 11e), interestingly, in this scenario implying that variation in transmission success between years is effectively entirely climate dependent, the only scenario/parameter combination outside of *Haemonchus* for this to occur.

Marshallagia (M) R_0 remains at 0 across all years, as in (2.0) (Figure12e). This is expected as if $R_0 < 0$ at the maximum saiga population size of 510,000 individuals, 300,000 individuals will likely always be insignificant for development in the absence of stronger climatic influence on transmission.

2.2) (2.0) with inclusion of “livestock” at fixed density in all ranges at all times of year.

The addition of alternative hosts across the entire year in each range massively increases R_0 in the lack of a Spring aggregation. In all years R_0 is positive for *Nematodirus* (NC) with a chilling requirement, *Nematodirus* with no chilling requirement (NNC) and *Marshallagia* (M) (Figure 10f, 11f, 12f). This is a stark contrast to (2.0) & (2.0). The effects of additional hosts are more pronounced when background host density is low year-round.

Investigating seasonality and livestock involvement

Additional graphs were also produced to highlight seasonality and transmission between years, and to further investigate the effects of livestock inclusion, these graphs and the associated written material have been included in the discussion for improved readability.

3.3 Fieldwork & Model Validation

Between faecal egg counts and collection and identification of adult worms from freshly deceased Saiga or livestock carcasses, no individuals of *H.contortus* were identified, eggs and adults of *N.sp* were found in low frequencies, no eggs of *M.marshalli* were found but adults were relatively numerous. Many other trichostrongylid eggs belonging to other GIN species were also discovered.

Adult worms transported to the UK had mixed success in identification, due to damage sustained in collection, travel or slide preparation, but those that could be identified could be done so easily. While there is no certainty, observations of adult worm structure compared to diagrams in Skrjabin *et al* (1954) suggest adult *Nematodirus* individuals found belong to species *N.dogieli* or *N.oiratianus.* All *Marshallagia* adults are thought to be *M.marshalli.*

Field studies in the 1980's and 1990's suggest the long term presence of *M.marshalli* and *N.sp,* particularly *N.gazellae,* although in some years there was failure to detect these species in the sampled carcasses or faecal egg counts. The presence of *H.contortus* and a wide diversity of *N.sp* in Betpak-dala in 1989 is explained by particulary high rainfall during this year. Presence of *H.contortus* in Betpak-dala could not be confirmed in late 1990's studies in Betpak-dala, and in both Betpak-dala & Ural from the 2017 field expedition findings.

Table 11) Presence/Absence of the chosen GIN species in the Kazakhstan Saiga populations of Betpak-dala and Ural, in historical studies and recent fieldwork. In the case of Nematodirus species, in historic literature, N.gazellae is present in all years from 1990-1993 in both Betpak-dala and Ural, while others are present inconsistently and at lower percentages (up to 30% host infection), the genus is therefore considered present.

	H.contortus	*N.sp*	*M.marshalli*
Betpak-Dala			
1989	20% prevalence	Present	80%
1990	Absent	Present	100%
1991	Absent	Present	50%
1992	Absent	Present	70%
1993	Absent	Present	Absent
Ural			
1990	20%	Present	100%
1991	40%	Present	100%
1992	Absent	Present	60%
1993	30%	Present	100%
(Priyadko *et al.* 1995)			
1997/1998 extractions from culled *Saiga* (Morgan 2003)	Absent	Present (0.75 – 54% prevalence depending on species)	Present (83% prevalence)
2017 Field Observations (This study)	Absent	Present	Present (Adults only)

Table 12) Identification of adult GIN worms collected from freshly deceased Saiga or livestock during 2017 fieldwork expedition to the Betpak-dala and Ural regions of Kazakhstan.

Carcass Identification	Number of Adults (Male, Female)	Genus of Males in Sample	
Saiga Ural 1	13, 21	*Nematodirus* *Marshallagia* *Haemonchus* *Other/Unidentifiable*	0 9 0 4
Saiga Ural 2	3, 10	*Nematodirus* *Marshallagia* *Haemonchus* *Other/Unidentifiable*	0 1 0 2
Saiga Betpak-dala	54, 69	*Nematodirus* *Marshallagia* *Haemonchus* *Other/Unidentifiable*	2 44 0 8
Goat Betpak-dala	8, 37	*Nematodirus* *Marshallagia* *Haemonchus* *Other/Unidentifiable*	8 0 0 0

Table 13) Faecal egg count data (n=117) from the 2017 fieldwork expedition to the Ural and Betpak-Dala regions of Kazakhstan. EPG = eggs per gram of

faecal matter. All EPG's refer to genus Nematodirus, as no Haemonchus nor Marshallagia eggs were found during the study.

Location	Sample Size	Average EPG	Max EPG	% Prevalence
Ural Site 1	37	2.30	20	19
Ural Site 2	29	1.03	10	17
Betpak-Dala Site 1	23	19.25	175	65
Betpak-Dala Site 2	28	7.60	30.0	64
Total	117	7.55	175	41.3

4.0 Discussion

Gastrointestinal nematode parasites exist in highly variable conditions, with climate strongly influencing the development, survival and availability of free-living stages and host presence often highly unpredictable. This variability reaches extreme levels in the saiga-nematode system, in which migratory hosts move over an environment that shows seasonal extremes in temperature and low average rainfall. Insights into whether and how parasites are able to persist in this system have implications for understanding threats to the health of endangered saiga populations, but also to grasp how parasite life history strategies have evolved to cope with these conditions. Climatic variation and host movement also feature in many farmed systems, and these insights might therefore be helpful to underpin sustainable parasite control in domestic livestock, and at the livestock-wildlife interface.

4.1 Main Findings

The main findings of modelling efforts in this study predict the success or failure of parasite genera under a variety of scenarios. We conclude that *Haemonchus contortus* is unsuited to the ecosystem parameterised in this model, whereas *Nematodirus sp.* and *Marshallagia marshalli* have the potential to survive and thrive assuming adequate host density.

4.1.1 Haemonchus

Under the conditions of this model, *Haemonchus contortus* fails to persist. At the macroenvironmental scale, the lack of consistent outweighing of evapotranspiration by precipitation, even over a time period as short as four days, prevents both the development to L1 within eggs and the development of free pre-infective larvae to the infective stage (O'Connor *et al.* 2008, Khadijah *et al.* 2013). Despite high mortality rates at extremes of temperature, the rapid development rate of this species means there is the potential for transmission were moisture not entirely limiting.

The theoretical failure of *H.contortus* to persist is supported by the total lack of this species in faecal egg counts and the intestinal tracts of saiga carcasses examined in

1997/1998 (Morgan 2003), and in 2017 (this study), however, the species was present in Kazakhstan in saiga as recently as 1989 (Priyadko *et al.* 1985) and in the 90's exclusively in sheep populations (Morgan 2003), meaning conditions must have been suitable for survival at some point, or other factors are at play.

H.contortus is known to survive at the very edge of its geographical range in the arid ecosystems of Saudi Arabia (El-Azazy 1995) and Mauritania (Jacquiet *et al,* 1995). In these cases, the ecosystem features a rainy season that provides reliable adequate precipitation for development for a portion of the year. High survival rates of arrested fourth stage larvae undergoing hypobiosis in the host allow *H.contortus* to escape the long dry season.

While Spring does typically bring an increase in precipitation in the Kazakh steppe, there is no specific "rainy season". It is possible that the resolution at which this model is applied is causing detail within the ecosystem to be lost. At the time of writing of this study, new research showed that microclimatic variables such as shade or long grass had a far greater effect of faecal moisture content and therefore development and migration of *H.contortus*, than macroclimatic temperature and moisture variables (Wang *et al.* 2018). Pockets of small areas suitable for *H.contortus* development and migration may be present throughout the saiga range.

Microclimatic variables such as soil moisture content may be impacted by anthropogenic factors such as mulching or irrigation for crop agriculture, therefore an additional effect of a growing human presence in Kazakhstan may have relevance for persistence of moisture dependent nematodes (Liu *et al.* 2008).

4.1.2 Nematodirus

Under most model scenarios in this study, parameters representing the *Nematodirus* genus resulted in successful theoretical transmission, with very high R_0 values with the inclusion of Spring host aggregation, and general maintenance of low transmission levels without this aggregation. This applies to different extents depending on the inclusion of a chilling factor in the model parameters (**Figure 10, Figure 11).** By comparing scenarios Saiga aggregation (1.0) vs Saiga aggregation with constant host population size (1.1) and No saiga aggregation (2.0) vs No saiga aggregation, with

constant host population size (2.1) for both *Nematodirus* with a chilling factor (NC) and *Nematoridurs* without a chilling factor (NNC), it is clear that host density is significantly more responsible for variation in R_0 than climatic factors, with only slight variation between in R_0 between years when host population size is constant.

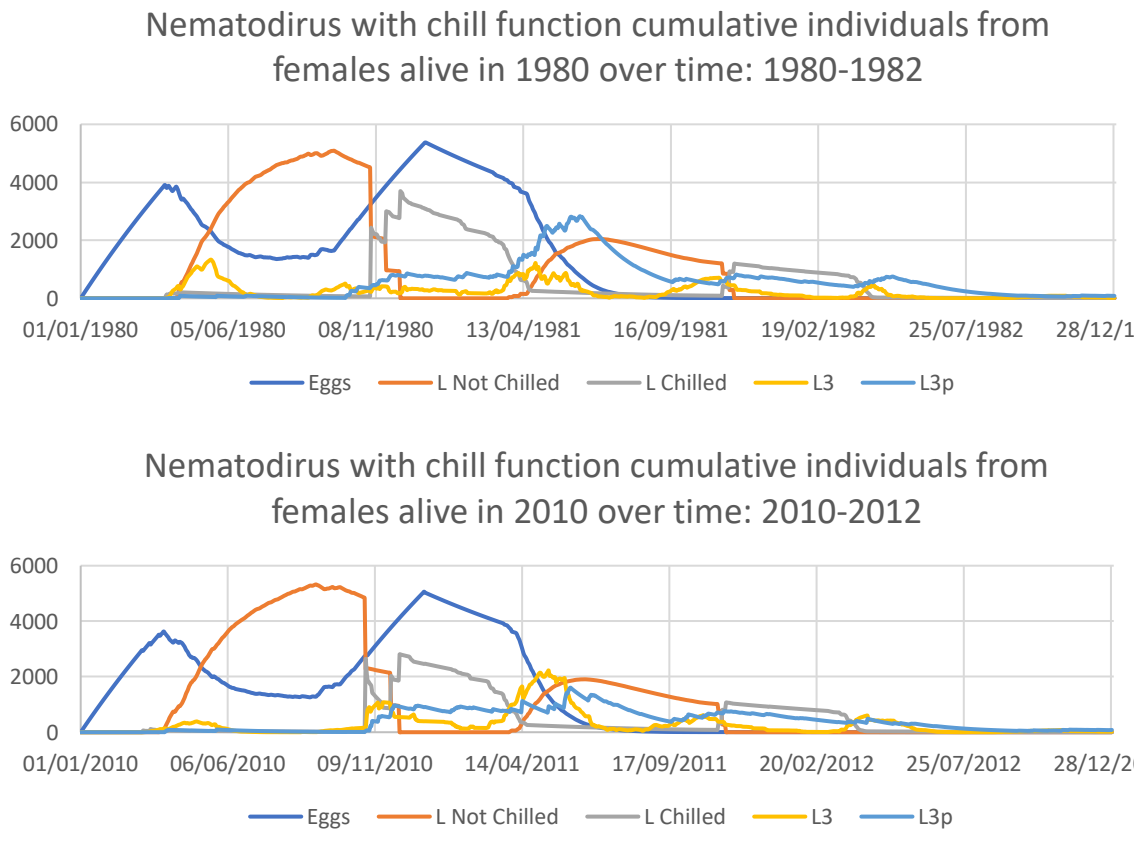
The low mortality rates incident on *Nematodirus* eggs, embryonated larval stages and free infective larvae over a large temperature range, throughout the year, allow adequate time for development and survivability until non-zero host presence (Morgan *et al.* 2005, Van Dijk & Morgan 2008, 2009). *Nematodirus* species have been present in *Betpak-dala* in high % prevalences across all historic literature (Morgan 2003, Priyadko *et al.* 1995) and were found both as adults collected from abomasa samples, and as eggs in faecal samples during the fieldwork involved in this study, supporting model conclusions that indicate persistence of this genus.

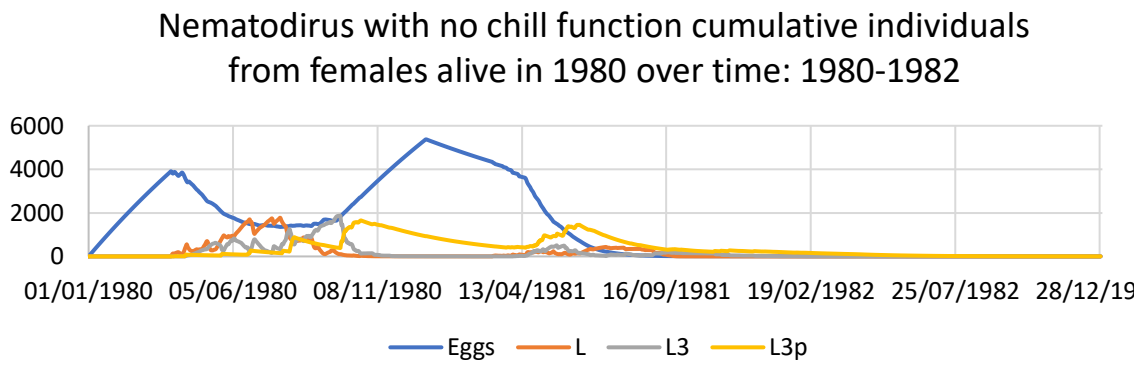
The significance of a bet-hedging strategy in this ecosystem can also be decided from this model and graphs of seasonality: **Figure 10, Figure 11 and Figure 13**. The key difference between chilling and non-chilling scenarios is the year in which the majority of embryonated eggs hatch and migrate to pasture L3. Without a chilling function, eggs begin development immediately following deposition. This is beneficial when development occurs quickly enough to allow infection of the population within a certain area of the migration route, within 2-4 months of deposition. If this does not occur however, L3p must wait for the hosts to return 6-8 months later, reducing in numbers at a climate dependent mortality rate for this duration. With a chilling function, larvae delay hatching until the required 28 days of average temperature below 4^{o}c (Van Dijk & Morgan 2008, 2009). Much like reality, this function better synchronises L3p availability to saiga host presence, as embryonated larvae sustain a low mortality rate over the course of a year, hatching at the beginning of spring as saiga arrive in their calving ground. This also partially explains why outputs of the models in this study show the majority of *Nematodirus* transmission occurs in the centre saiga range (Figure 15).

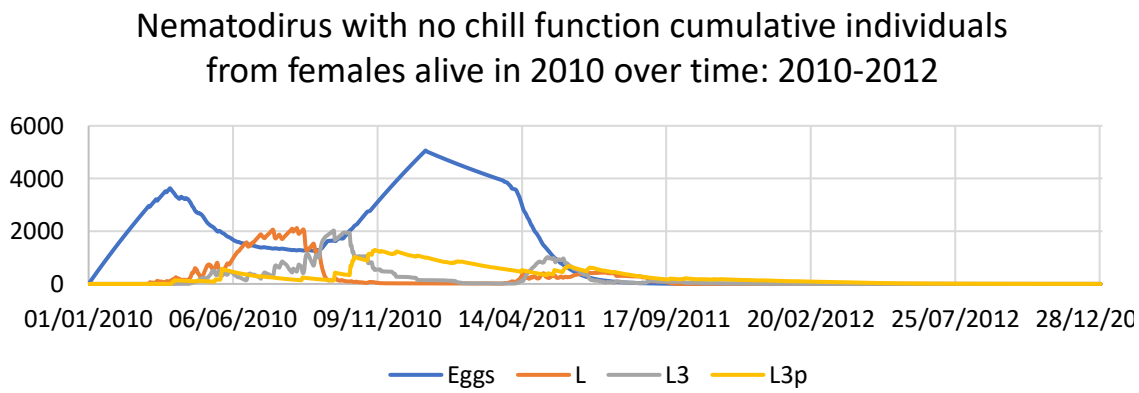
While in this model it is predicted that bet-hedging results in higher reproductive success, the caveat must be acknowledged that in reality the return of saiga to the same location between years is never guaranteed as calving sites vary between years

(Bekenov *et al.* 1998, Singh *et al.* 2011). While a bet-hedging in a portion of offspring may be useful due to the unpredictability of host availability in this system (Beaumont *et al.* 2009, Van Dijk & Morgan 2010), in reality, bet-hedging could cause *Nematodirus* eggs to miss opportunity for transmission completely if saiga do not return to the location of egg deposition in years two and three following deposition. This may explain why *Nematodirus* species infective of Saiga such as *N.gazellae* and *N.spathiger* do not exhibit bet-hedging.

Figure 13) An investigation into seasonality - Tracking the individuals produced by a single adult N.sp in the time period of one year, over all three saiga ranges, over the three year period spent by this population of theoretical offspring in the external environment..







4.1.3 Marshallagia

As described, *Marshallagia* eggs accumulate under a high survival rate, particularly at cold temperatures, until ideal conditions for development and hatching arise, at which point development from egg to L3 is rapid for as long as these conditions persist (Carlsson *et al.* 2013) (***Figure 14***).

High survival rates of L3 and pasture L3 due to protection gained from the conversion of L3 sheath, allow survival of infective larvae through the second year following deposition, although effectively all L3p had been lost to mortality or transmission by year 3 (Morgan *et al.* 2007, Carlsson *et al.* 2012).

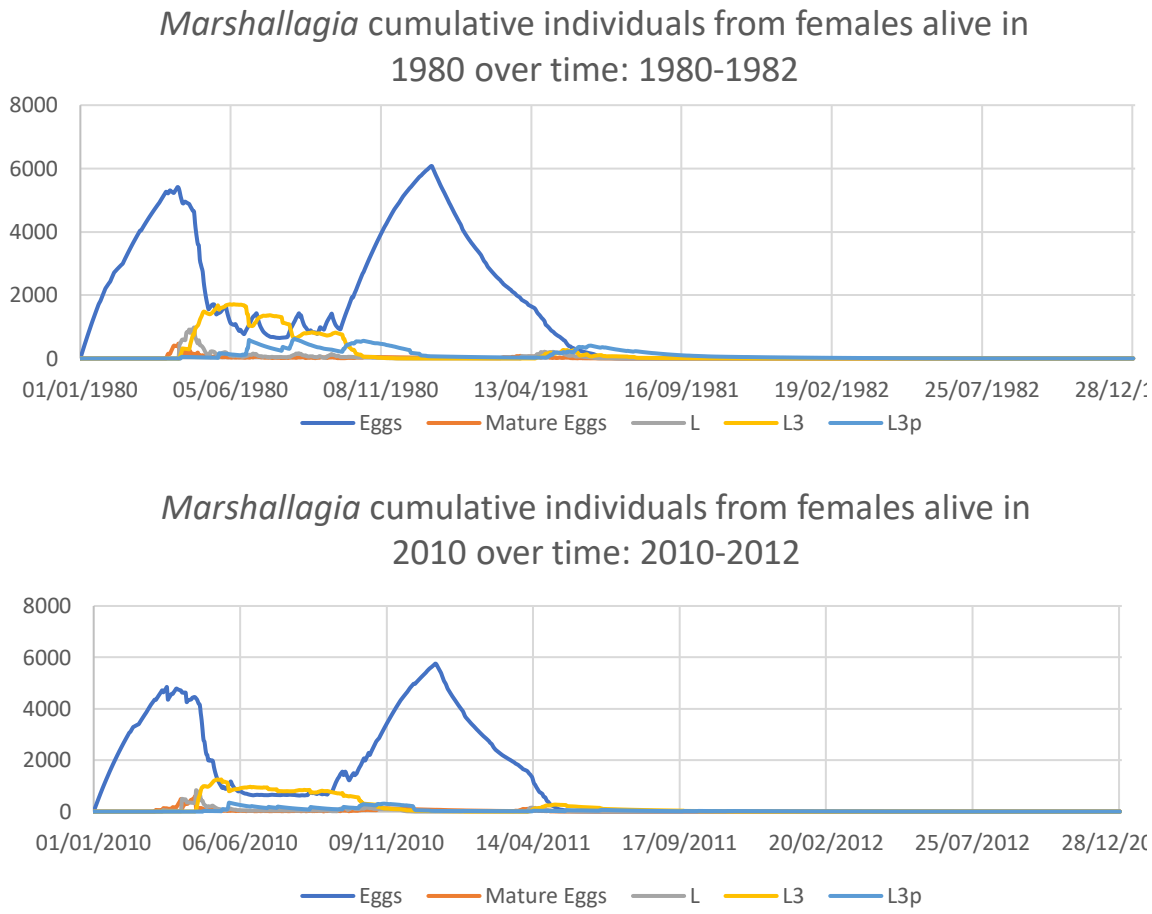
Similar to *Nematodirus,* there is a reliance on spring conditions and high host density for transmission success, which is logical as peak *Marshallagia* burdens are known to occur during summer (Irvine *et al.* 2000). It is known that temperatures below freezing are not necessarily lethal for *M.marshalli* but development cannot occur during the winter (Carlsson *et al.* 2012) and the absence of development from May to September

in arid steppe studies (Meradi *et al.* 2011) suggests spring and autumn are the only window for *Marshallagia* transmission.

The dependence of high host density for transmission is obvious in the absence of R_0>1 in any year without spring aggregation, even with host population size fixed (scenarios 2.0, 2.1, ***Figure 12de*****)**. Despite a fecundity double that of *Nematodirus* and a faster development rate of intermediate larval stages, less L3p are available in synchrony with host presence, likely due to higher mortality rates of eggs and early infective larvae.

The presence of *Marshallagia* adults in historic studies and in the fieldwork results of this study support model findings that this species can persist under the present climatic conditions. Further, modelling efforts in this study suggest that host density is adequate for at least a portion of the year to allow adequate *M.marshalli* transmission.

Figure 14) An investigation into seasonality - Tracking the individuals produced by a single adult M.marshalli in the time period of one year, over all three saiga ranges, over the three year period spent by this population of theoretical offspring in the external environment.



4.1.4 Alternative Host Presence

Livestock scenarios warrant further investigation. The key question and reasoning for this scenario is "can alternative hosts sustain R_0 when the presence, abundance or density of primary hosts is otherwise too low to do so?".

Saiga host population sizes were at their lowest from 2002-2004, therefore graphs were plotted of the monthly average R_0 of a female depositing eggs during 2002, for *Nematodirus* with a chilling factor (NC), *Nematodirus* with no chilling factor (NNC) and *Marshallagia* (M), comparing saiga aggregation/saiga aggregation with livestock

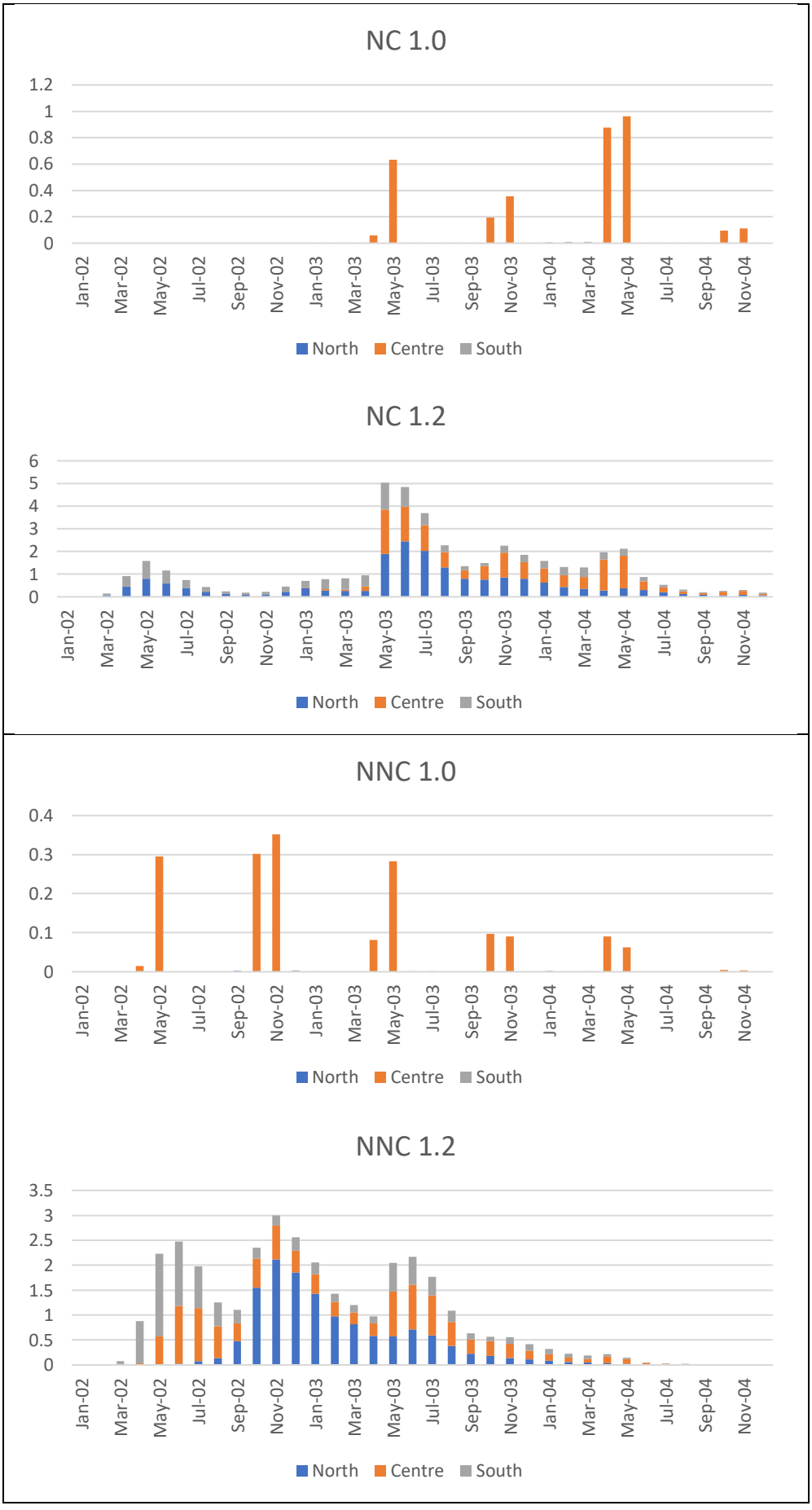
inclusion (1.0/1.2) and no saiga aggregation/no saiga aggregation, with livestock inclusion (2.0/2.2) scenarios.

Firstly, a one-year examination of the 1.0/2.0 scenarios reveal clearly the previously discussed reliance of pasture larvae on aggregation in the central region for high transmission potential. The failure of any species to sustain transmission in the low host densities of 2.0 scenarios is also clearly visible.

For NC, NNC and M, the presence of alternative hosts at all times of year greatly increased the R_0 of the female worm that deposited eggs during 2002. With alternative hosts in all ranges, R_0 for the 2002 female was >1 in every scenario. Transmission potential in the northern and southern ranges was frequently on par with that of the central range, in contrast to saiga only scenarios.

The implications of successful transmission to an alternative host of fixed location and population size is the generation of reservoir populations. In scenarios where climatic factors result in a mismatch between the availability of pasture L3 and the presence of susceptible hosts, the ingestion of this pasture L3 by a fixed location alternative host may allow continued persistence of this species until the primary host returns (Kao *et al.* 2000, Fenton *et al.* 2015). In this Kazakhstan-based model the alternative host scenarios represent livestock presence. Previous models have considered transmission of GIN from livestock to Saiga (Morgan *et al.* 2007). At present this study supports the transmission of GIN from Saiga to livestock but cannot confirm the opposite in its current form. Future work is recommended to convert the model framework used in this study to allow egg-input from non-saiga hosts, to add further data in support of this research.

Figure 15) (Following 3 pages) Graph Series showing monthly average R0 across scenarios for Nematodirus* with a chilling factor (NC), *Nematodirus* with no chilling factor (NNC) and *Marshallagia* (M)*parameters. Graphs are stacked by the area of the saiga range in which successful transmission is occurring.


NC 1.0
1.2
1
0.8
0.6
0.4
0.2
0
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
Mar-03
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Sep-04
Nov-04
North
Centre
South
NC 1.2
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4
3
2
1
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May-04
Jul-04
Sep-04
Nov-04
North
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South
NNC 1.0
0.4
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Jan-02
Mar-02
May-02
Jul-02
Sep-02
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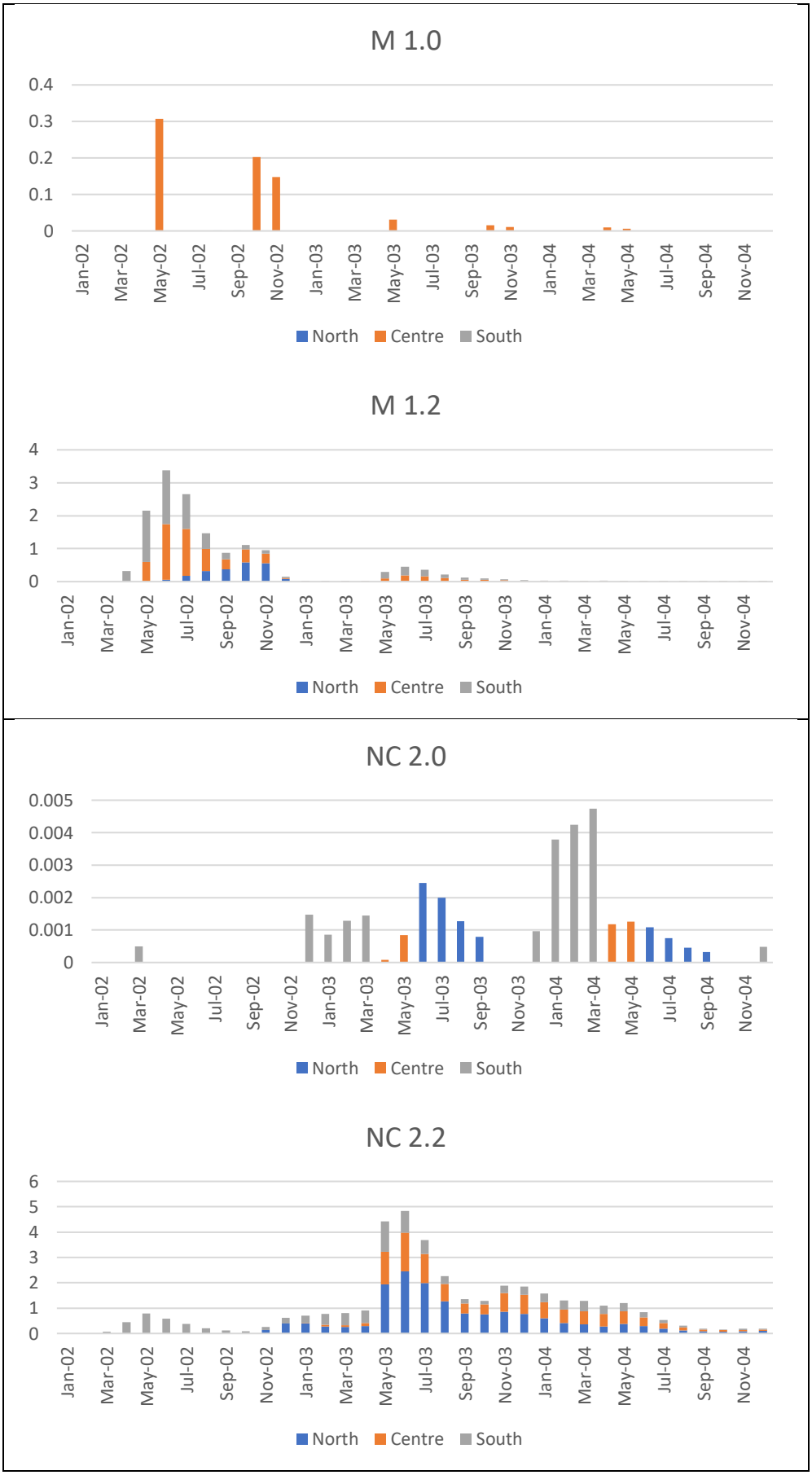
M 1.0
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May-03
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Nov-03
Jan-04
Mar-04
May-04
Jul-04
Sep-04
Nov-04
North
Centre
South
M 1.2
4
3
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Jan-02
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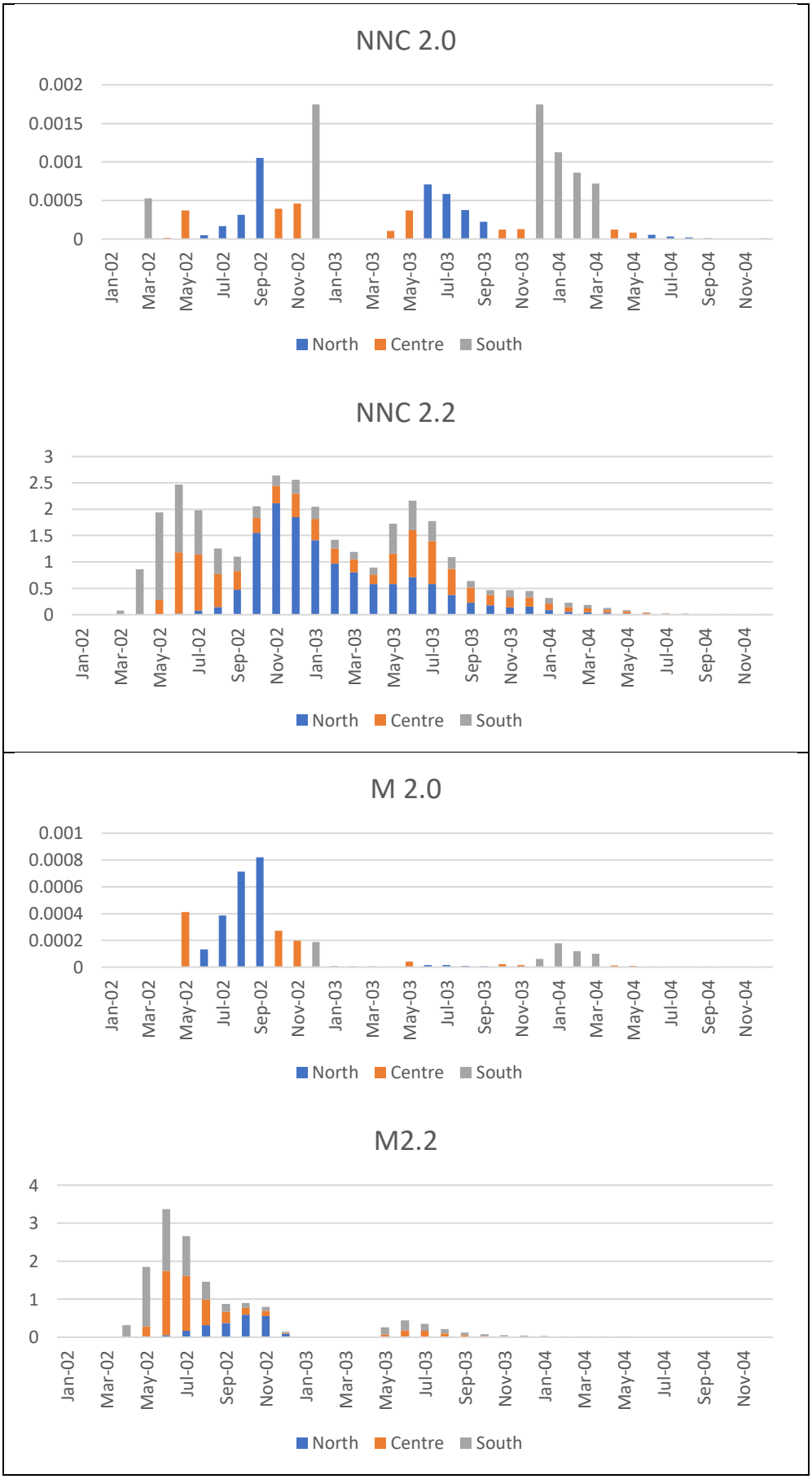
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4.1.5 Summary

With *Nematodirus* and *Marshallagia* generally favoured to persist in this region over *Haemonchus,* it is clear that this climatically and demographically variable system requires the traits typical of a K-selected population – high investment in offspring and high individual survival rate, as opposed to the opposite traits of r-selected populations. In this system, GIN at stages of the external life cycle must endure the harsh climatic conditions long enough for development to the infective stage and host presence to occur.

Host density is critical for transmission success and has a greater effect on transmission potential than climatic variables other than in *Haemonchus contortus.* Should host density fall too low over such vast area, rate of pasture L3 intake becomes too low for positive R_0 to be accumulated within timeframe of survival of offspring deposited by a single female over the course of a year.

4.2 Future Threats & Other Contexts

Further collapse of Saiga due to poaching or pasteurellosis

As shown in this study, a dramatic fall in saiga host density is likely to significantly reduce transmission potential across all of the GIN species studied when considering saiga hosts alone, to the point that transmission is inadequate for persistence.
Conservation efforts in the early 2000's were successful in raising saiga population sizes across their range (Singh *et al.* 2010), however authors recognise the impossibility of controlling outbreaks of *Pasteurella multocida* (Kock *et al.* 2018).
The theoretical maintenance of parasite genera in reservoir livestock populations throughout the saiga range has the potential to increase parasite burden in saiga if the future population recovers from further decline or mass-mortality events (Kao *et al.* 2000, Fenton *et al.* 2015).

Return of saiga population size to a sustainable historic level

Model outputs have suggested that a host population size of approximately 500,000 individuals is sufficient for parasite persistence in *N.sp* and *M.marshalli*, even when hosts are considered to be distributed uniformly across their maximum range size. Therefore, a return of saiga population size to historic levels may greatly increase the ability of parasite genera to persist solely using saiga as a host species. Changes in population size are not predicted to have any noticeable impact on *Haemonchus contortus* persistence without change in climatic variables.

Cessation of migration

Climate warming is causing a rise in vegetative productivity, leading to earlier food availability in central and northern saiga ranges, therefore reducing the necessity of saiga to move out of northern range (Robinson 2000, correspondence, Singh *et al.* 2010, 2011.) In the theoretical scenario that saiga occupy one range for the full duration of the year, effects on transmission will be similar to that of alternative host presence, where R_0 is increased due to greater host availability. The greater average annual precipitation in the northern range has implications for the transmission of moisture dependent GIN species such as *Haemonchus contortus,* if saiga are present in this range outside of the dry summer months. In the absence of food pressure for migration, pressure of increased parasite burden may select for the maintenance of some movement over a smaller scale (Hall *et al.* 2016).

Restocking of the saiga range with livestock

As shown in this study, alternative host presence greatly increases parasite transmission in this system. While livestock has been restricted to near villages in recent times, growth in human population size and an increasing demand for meat may drive the spread of livestock agriculture throughout the saiga range (Lundervold 2001). A greater livestock population size and distribution increases the probability of saiga and livestock sharing pasture for periods of the year and therefore an increased probability of the development of GIN reservoir populations and transmission between

hosts sharing pasture (Kao *et al.* 2000). While this would theoretically remove selection for life-history strategies that cope with infrequent host presence, these adaptations such as hypobiosis and bet-hedging also diminish the effects of extreme environmental conditions and may be selected to remain. These factors are difficult to disentangle when predicting future parasite adaptation.

Climate Change

While in this study the effects of climatic variation were found to be less important than host density for 2/3 genera, over time changes might have a larger effect.

The life cycles of the discussed species use windows of development under ideal climatic conditions timed with host presence to facilitate transmission. Climate change has the ability to disturb this timing, causing asynchrony and a reduction in parasite presence, or it may improve conditions for transmission, development or mortality at certain periods of the year (Cizauskas *et al.* 2017).

The predicted increased aridity of the region over time (Turco *et al.* 2015) is likely to increase the unfavourability of conditions for *Haemonchus* transmission (Rose *et al.* 2016). The adaptations of *Marshallagia* and *Nematodirus* may allow continued persistence despite this.

Carlsson *et al.* (2012) predicted increased winter temperature could lead to increased *M.marshalli* burdens as development rates increase and mortality rates decrease, on top of *M.marshalli*'s cold resistance already making it capable of transmission over winter months. However, increased summer temperatures may widen the portion of the year that parasite larvae must endure with no chance of transmission.

The high resistance to temperature extremes and desiccation observed in *Nematodirus* imply that this genus is the most likely to persist in the face of an ever more extreme climate in this climate change hotspot (Van Dijk & Morgan *et al.* 2008, 2009)

Parasites may adapt to variations in any of the discussed scenarios and factors over time within this environment. The evidence for this is the variation of life-history traits

within species over the huge geographic ranges of the discussed species – variation in *Haemonchus* hypobiosis, in *Nematodirus* bet-hedging, and the temperature thresholds for development in *Marshallagia marshalli.* Given gradual changes adaptation may allow persistence of any of the discussed genera, although as shown in this model, climatic and particularly demographic factors can change quickly enough to drive local extinction of species.

The findings of this study have implications for any host-parasite scenario involving “migration”. Even if this migration is anthropogenically driven. For example, if parasites are adapted to survive and persist in conditions of periodic host absence, then they may be pre-adapted to evasive strategies such as the rotation of pasture within and between years, in livestock agriculture. These factors should be taken into account when attempting to tackle nematode induced disease.

4.3 Concluding Statements

This study has demonstrated the usefulness of a mathematical model to investigate the effects of demographic and climatic stages on development and mortality at different stages of the GIN external life-cycle.

In this system, parasite life history strategy is predicted to trend towards that of K-selected life-history, where high survival rates, high investment in offspring and specific adaptations to persist under non-ideal environmental conditions allow the overall persistence of GIN species in this system. High variation in host-availability is the main cause of parasite success/failure within species with high survivability in this system, and therefore adaptations against this problem are most likely to be selected for.

The future of the presence of individual GIN genera in this system will vary with the climatic and demographic factors incident on this system in coming years. This study has made inferences of the consequences of these potential future changes in the

variables affecting transmission, that may contribute to future conservation initiatives or sustainable livestock maintenance strategies.

Suggestions for further research related to these efforts include –

1) Laboratory experiments on the eggs and larval stages of *M.marshalli* and *N.gazellae* isolates, as well as the application of a sensitivity analysis to correct estimates, would confirm or modify current model parameterisation that is based partly on estimation. This is particularly crucial for *M.marshalli* as the effects of climatic variables on this species remains poorly understood. This would also provide much needed information on the requirement of chilling for hatching and development of *N.gazellae.*

2) Further study would seek to instigate regular parasite monitoring in the Kazakhstan saiga/livestock system, alongside current ecological surveillance efforts to build a multi-year time series of parasite prevalence. This would increase model accuracy over time and provide much needed information on parasite prevalence in ecosystem.

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